University of Alberta

Distribution of Pentosans in Barley Pearling Flour Fractions

by

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To my family, my mother María Guadalupe Cornejo de Olmos, my father Jorge Eduardo Olmos González and my brother, Cesar Eduardo Olmos Cornejo for their support and understanding. To all my friends in Canada and Mexico, especially the friends I met at the University of Alberta who encouraged and motivated me to finish this thesis.

ABSTRACT

Pentosans contribute to the cell wall structure in wheat, rye and barley. These dietary fibre components were extracted from Candle pearling flour in four sets: i) different initial alkali concentrations (0.04M-0.2M NaOH) at 55°C [pH 9 and 11]; ii) water or alkali (0.1M NaOH) extraction at different temperatures (23, 55 and 90°C) and pH (6 and 11); iii) use of α-amylase, fungal protease or ultrasound treatment at 55°C and pH 5.5; iv) water or 0.0375M NaOH at 55°C, pH 5.5 and 11, and enzyme-ultrasound combination treatment. Soluble and insoluble fractions obtained were quantified, examined with scanning electron microscopy and analyzed for composition, yield and recoveries. The majority of pentosans was not solubilized and ended up in the insoluble fraction, except for the high alkali conditions. Samples treated with ultrasound presented higher pentosan content with better disruption of the bran tissues. These findings contribute to the value-added processing initiatives for barley.

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ABBREVIATIONS

AACC: American Association of Cereal Chemists

DD: Distilled De-ionized

DF: Distrito Federal

EU: European Union

FDA: Food and Drug Administration

FTA: Free Trade Agreement

GLM: General Linear Model

Hg/Ha: Hectogram/Hectare

HSD: Honestly Significantly Different

Mt: Metric tonnes

NAFTA: North American Free Trade Agreement

NSP: Non Starch Polysaccharides

PF: Pearling flour

SAGARPA: Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación

SAS: Statistical Analysis System

SEM: Scanning Electron Microscopy

SIAP: Servicio de Información y Estadística Agroalimentaria y Pesquera

T: Traces

U.S.: United States

USDA: United States Department of Agriculture

(w/w) d.b.: (weight/weight) dry basis

1. INTRODUCTION AND THESIS OBJECTIVES

The cereal hemicelluloses have valuable properties (silage digestibility, bread making quality, insect resistance, and brewing properties) but the development of appropriate processes for their isolation at a commercial level has not been evaluated.

The carbohydrates in cereals are classified into simple sugars, starch and non-starch polysaccharides (NSP). In barley, pentosans make up an important part of NSP along with β-glucan. Pentosans are comprised of a small but representative group of polysaccharides called arabinoxylans, which constitute the primary material of cell wall structure in some cereals, such as wheat and rye, but in others like oats and barley are found to be mixed with β-glucan. In addition, arabinoxylans maintain tissue integrity and improve water transportation, including solutes and nutrients.

Cereal pentosans are classified as anti-nutrients in the feed industry due to their high viscosity, causing problems of assimilation of nutrients. The brewing industry is also affected by these properties, but further research is needed for a better understanding of the changes and interactions of pentosans during their processing.

Even though cereal pentosans present some undesirable properties especially for the feed and brewing industries, from the human nutrition point of view, they are a valuable component of dietary fibre. However, the literature lacks information on isolation, functional properties and potential use as a fibre ingredient in a variety of food product applications.

Dietary fibre consumption in North America is too low; according to the U.S. government, the people in the United States are consuming only 30% of their daily needs.

It is well known that inadequate fibre intake is associated with a range of potential health issues and may be a major part of the obesity epidemic in this country. In addition, fibre is an essential component of a healthy lifestyle since it reduces the risk of colon cancer, heart disease and type II diabetes.

Even though wheat arabinoxylans have been analyzed and studied in depth, not enough research has been performed with other cereals of similar compositions, such as rye, barley, oat and maize. There are reasons to believe that arabinoxylans of other cereals could function in ways similar to those found in wheat, but further research is needed to explain the true nature of various processes, involving extraction, characterization and applications in the food industry as a functional ingredient.

In terms of barley cell wall components, most of the research studies have focused on the extraction and characterization of β-glucan, a soluble fibre component located in the endosperm cell walls, showing great potential as a nutraceutical (refers to phytochemicals or natural, bioactive compounds that have health promoting, disease preventing or medicinal properties). However, there are no studies on barley pentosans at extraction conditions relevant to fractionation processes developed to date (for β-glucan and protein isolation) targeting the application of the fractions obtained in food products. Furthermore, simultaneous evaluation of β-glucan/protein/pentosan distributions under such conditions has not been reported.

In Canada, specifically in the province of Alberta, barley is one of the major crops produced. Around the world, food applications of barley have been limited with the majority of barley produced being utilized in animal feed and brewing industries. This

situation leads to an important option ready to be explored. Hence, the development of new approaches for food applications presents a major advantage for producers and processors, in an effort to diversify their product between different value-added markets.

One such approach may be the isolation of pentosans from barley pearling flour, which is a by-product of the conventional pearling operation, with the ultimate goal of their utilization as a fibre ingredient in a variety of food product applications.

Therefore, the overall objective of this thesis was to enhance the utilization of barley, based on the isolation of high-value functional ingredients such as pentosans. The specific objectives of this thesis were:

1. to determine the effects of solvent type, pH, alkali concentration and temperature on the composition (pentosans, β-glucan and protein) and yield of the soluble and insoluble fractions of the pearling flour of CDC Candle barley (Chapter 3), and
2. to determine the effects of different enzyme and ultrasound treatments to maximize the concentration of insoluble pentosans from the pearling flour of CDC Candle barley (Chapter 4).

2. LITERATURE REVIEW

2.1. Barley

Hordeum vulgare, commonly known as barley, is the fourth most important cereal crop in the world, just after wheat, maize and rice. World production of barley represents about 40% of wheat production. Historically, barley is one of the first crops adopted and domesticated by humans and grows in a wide range of environments but it is mainly produced under unfavorable climates for other major cereals. Barley possesses unique characteristics that have made it an important grain in the past and present, with wide adaptation and use as feed and food grain as well as in malting and brewing processes.

Barley was found for the first time around 5,000 to 7,000 B.C. (Clark 1967; Harlan 1976), but some researchers have shown the existence of barley even 10,000 years ago in the Nile valley (Wendorf et al 1979). Also, the same historians state that at the time, barley was the most abundant and cheapest grain in the ancient Near East (from present-day Israel, Jordan and southern Anatolia to western Iran).

Previous to the studies of Helbaek in the period 1960-1970, different studies discussed the presence of barley in the older times and the archeological varieties discovered, confirming the presence of the six-rowed type over the two-rowed types (Zohary 1973). After this and based on Helbaek's studies, Zohary (1973) confirmed that the old barley remains (7,000 and 6,000 B.C) were mainly two-rowed forms. In addition, Evans (1968) and Harlan (1976) confirmed and concluded that the evolution and domestication of barley present a certain level of doubt. Evidence suggested that the most

recent and immediate ancestor of barley was *H. vulgare spontaneum*, a two-rowed wild type, revealing at the same time that the six-rowed type was produced by the action of mutations and hybridizations (Harlan 1976).

In recent years, barley has evolved and adapted, bringing new importance into the agriculture. Just in the past 20 years, the production of barley has increased faster than even wheat or rice, showing high yields.

2.2. The importance of barley in the world

The discussion on value-added processing and further utilization of barley targeting increased human consumption requires and understanding of the current production levels, its distribution around the world and current uses. Therefore, potential expanded barley utilization brings the economical interaction between countries and the agreements created for trading into the analysis. Thus, barley production, trade and utilization in the world are presented in this section and those for the countries involved in The North America Free Trade Agreement (NAFTA) are discussed in the next section (section 2.3).

Barley is one of the top four cereals cultivated in many places around the world and represents a significant proportion of the total cereals production. Barley production, like wheat in the western countries and rice in the eastern world, is often a cultural consideration, rather than an economical or nutritional condition, as well barley is grown

where other cereals do not grow well due to altitude, latitude, low rainfall or soil salinity (Wendorf et al 1979).

In Europe, for the last decades barley has been clearly supported by the governments (through subsidies), resulting in a constant production of barley and malt and therefore, making the European Union (EU) one of the largest traders in the world (Table 2.1). As a result of this, U.S. exports have been declining since the 80s, when the effect of European subsidies was felt by the U.S. producers, leading to a loss in market share. The U.S. produces only 4-5% of the 130-155 million metric tonnes of the total world production of barley, and the U.S. is one of the top five barley exporters in the world. The EU, Russia, Canada and Australia comprise almost the entire balance of trade (Fig. 2.1).

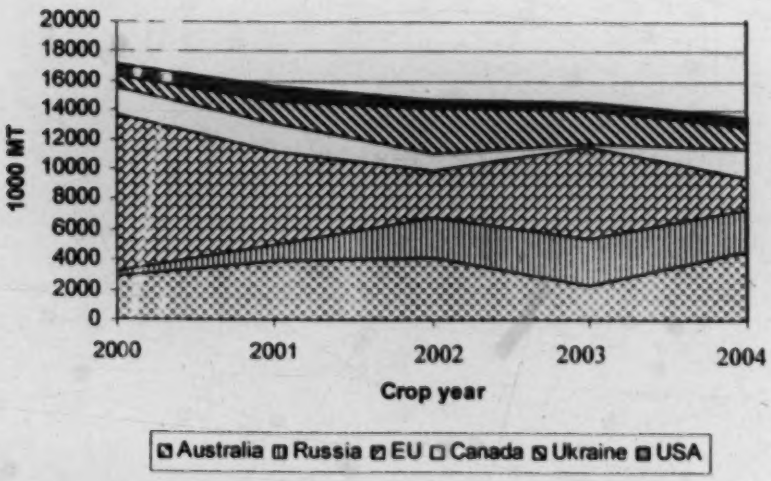

Figure 2.1. World barley trade (Source: USDA, 1990-2003)

Table 2.1. Barley production (thousand tonnes) in the major producing countries for the period 1993-2002

Year	European Union (EU)	Russian Federation	Canada	Ukraine	USA	Turkey	Australia	China (P.R.C.)	Poland	Kazakhstan	Others	Total
1993	47039	26900	12972	13550	8666	7300	6956	4327	3300	7149	31019	169178
1994	43687	27000	11692	14508	8162	6500	2913	4411	2686	5497	33680	160736
1995	43713	15800	13033	9633	7824	6900	5823	4089	3278	2178	29392	141663
1996	51716	15900	15562	5725	8544	7200	6696	4000	3437	2700	32140	153620
1997	52608	20800	13527	7407	7835	7300	6482	4000	3866	2670	27760	154255
1998	51907	9800	12709	5870	7667	7500	5987	2656	3612	1100	27022	135830
1999	48929	10600	13196	6425	6103	6600	5032	2970	3401	2250	22229	127735
2000	51565	14100	13229	6872	6939	7400	6743	2646	2783	1675	23990	141788
2001	48090	19500	10846	10186	5430	6900	8423	2893	3300	2200	23990	241788
2002	48023	18700	7489	10350	4933	7400	3713	2470	3369	2200	24144	132791
Average	48728	17910	12426	9053	7210	7100	5877	3446	3306	2962	27068	145085

Sources: Canada (Statistics Canada 2004), all other countries/total (United States Department of Agriculture, USDA 1990-2003)

Saudi Arabia, Japan and China are the largest importers of barley. The barley imported by Saudi Arabia is used for feed. Japan imports barley for both feed and malt production. A considerable percentage of the world and American exports are in the form of malt. Meanwhile, Japan and Mexico remain as top customers for the barley produced in the U.S and Canada. Canadian barley (malt) is mainly exported to Central and South America and South Africa.

2.3. Barley production in the countries under the NAFTA agreement

The North American Free Trade Agreement (NAFTA) came into effect on January 1, 1994. The main objective of this agreement is to eliminate the commercial barriers for trade and investments between Canada, Mexico and U.S., including the barriers for agricultural products. The NAFTA agreement has eliminated a considerable portion of barriers between the U.S. and Mexico, some of which were applied immediately; whereas others will be implemented over time, reaching its peak in the year 2008. On the other hand, Canadians and Americans incorporated the Free Trade Agreement (FTA), established in 1989, into the NAFTA agreement with the elimination of all agricultural tariffs by January 1, 1998. Canadians and Mexicans reached bilateral agreements that were incorporated into NAFTA, in a different way, working towards eliminating most tariffs in periods of 5, 10 or 15 years for agricultural products, especially dairy, poultry, eggs and sugar.

The area harvested, yield, production and barley seed used for the members of NAFTA, averaged for the 5 year period 1999-2003 are shown in Table 2.2.

Table 2.2. Barley producing countries of NAFTA in the period 1999-2003

	Area Harvested (Ha)	Yield (Hg/Ha)	Production (Mt)	Seed (Mt)
Canada	4,004,175.00	27,665.50	11,189,800.00	426,750.00
U.S.	1,858,085.00	31,389.00	5,851,500.00	189,414.00
Mexico	277,601.00	23,792.25	666,384.25	16,962.50

Source: FAO (2004)

2.3.1. Barley production in Canada

After wheat, barley is the second ranking cereal in Canada and is grown mainly in the Prairie Provinces. The provinces with the highest production of barley are Alberta, Saskatchewan and Manitoba, representing 90% of the total production of the nation. Of the total production, 90% is kept on the farms to be sold as feed or seed and the rest is used for malting and malt exports (Statistics Canada, 2004) (Fig. 2.2).

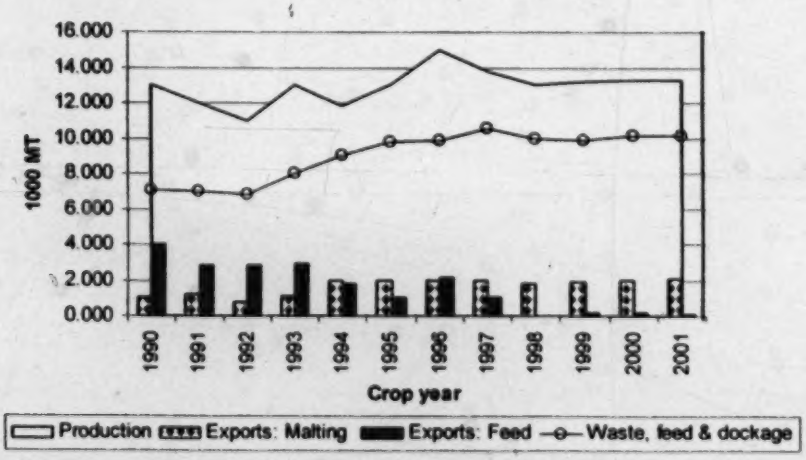

Figure 2.2. Barley in Canada: Production and trading

Source: Canadian Grain Commission (2001)

2.3.2. Barley production in the United States of America

In the U.S., barley is the third major feed crop produced, after corn and sorghum. The five top-ranking barley-producing states are located in the northern part of the U.S., comprised of North Dakota, Montana, Idaho, Minnesota and Washington. The production of the five top states represented 75% of the total barley produced by the nation (USDA, 1990-2003). During 1985-1990, 58% of barley produced was used for feed, 40% was used for malting and brewing with only 2% used for food. For the same period, the value-added for the total production of barley was approximately $8.2 billion USD.

Between 1999 and 2003, an average of 2 million hectares was planted in the country and although this is less than 2% of the total crop area in the U.S., the country is the seventh largest barley producing country in the world (Fig. 2.3).

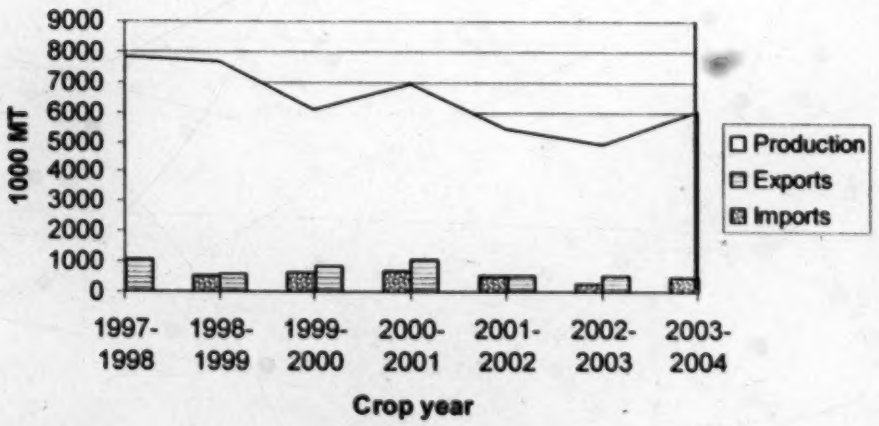

Figure 2.3. Barley in the U.S: Production and trading
Source: U.S. Grain Council (2003)

2.3.3. Barley production in Mexico

The situation of barley in Mexico is different compared to the other members of NAFTA. In Mexico, barley ranks fifth. The comparison between barley and corn productions in the country is not proportional. While corn represents the main grain produced in the country with 24,310,000 tonnes (SIAP, SAGARPA 2004), barley represents only 1.0% of the total grain production. The major barley producer states of the country are Hidalgo, Michoacan and Puebla and their production represents 85% of the total. Their major use is for malt processing and in minor proportions for animal feed. Due to the insufficient production of barley, Mexico has imported approximately 500,000 tonnes every year from U.S. and Canada (SEGARPA 2004) (Fig. 2.4).

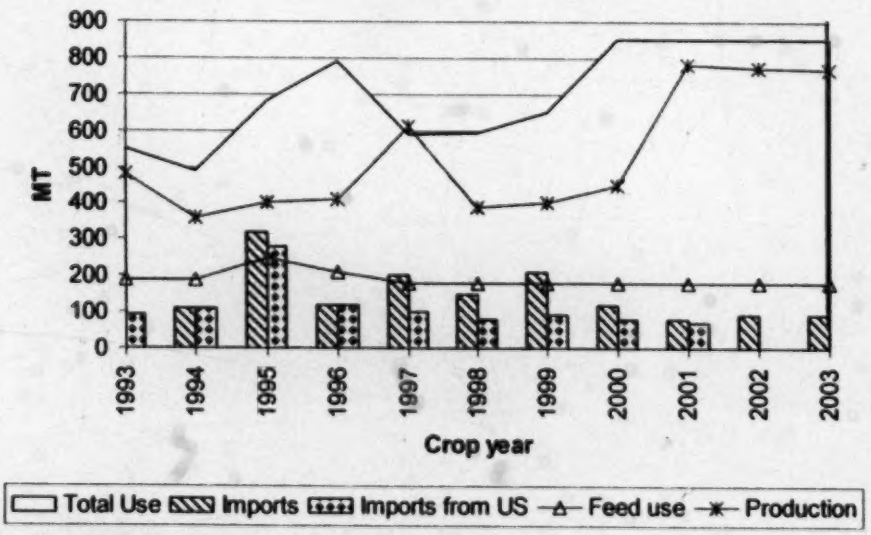

Figure 2.4. Barley in Mexico: Production and Trading
Source: U.S. Grain Council (2003)

2.4. Structure and overall composition of barley

Barley grain is considered a caryopsis and consists of the pericarp, integuments, aleurone layer, the endosperm, germ or embryo (attached to the dorsal side of caryopsis) and surrounded by two main portions: lemma and the palea (hulls) (Fig. 2.5).

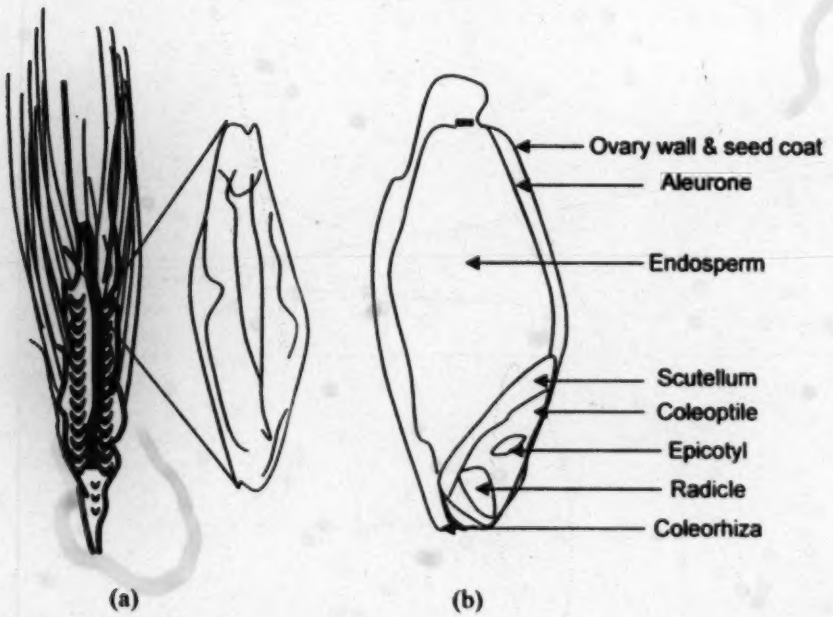

Figure 2.5. Barley spike: a) grain and b) grain structure (Adapted from Rasmusson, 1985)

The structure of the grain is formed by several layers; all of which have specific functions and, at the same time, provide support in the composition and final form of the grain. The first layer of the grain is the pericarp, which is the protective coat of the kernel and is formed by the ovary wall. Different from the pericarp, and responsible for the

protection of the nucellus of the ovule are the integuments, an outer cell layer located in the seed coat. An important component for the nutrition of the grain, especially during germination is the endosperm. The endosperm is responsible for providing the nutritious tissue for the development of the embryo. Among the other outer layers of the grain, the outermost layer of the endosperm is the aleurone. This layer contains a combination of protein bodies and enzymes that are involved in the grain digestion during the process of germination, providing the energy and necessary elements to hydrolyze those components located in the endosperm.

The embryo, located at the end of the caryopsis, plays an important part in the formation of the young plant. Inside the embryo are small structures that have an active participation during the germination, providing protection and helping the new plant to emerge and grow.

Kernels have been also studied to explain the differences in characteristics from one cultivar to another and to understand the variations between them and their advantages during growth and development (Reid et al 1971). One of the most recognized characteristics between kernels is whether they are husked (covered) kernels or naked (hull-less) kernels. This condition is determined by a small variation in the lemma and palea during maturation, dictating whether the hull firmly adheres to the grain or simply falls off during harvest. The kernel has been important for the identification of barley varieties since there are visible differences between the two and the six-rowed varieties. While the two-rowed barley cultivar has symmetrical kernels and their sizes are

larger in the middle of the spike and smaller close to the tip, the six-rowed group shows twisted kernels with asymmetrical shapes (Fig. 2.6).

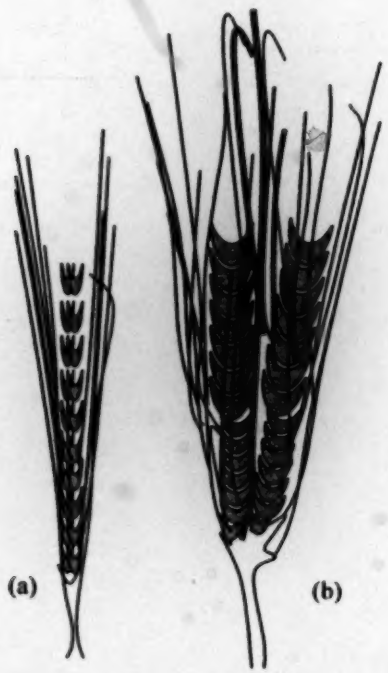

Figure 2.6. Barley spike: a) six-rowed, b) two-rowed (Adapted from Rasmusson, 1985)

The barley (*Hordeum vulgare L.*) kernel, like other cereal grains, contains carbohydrates (80%), proteins (10%), lipids (3%), minerals (2%), vitamins and other minor components (~5%) (Brandt 1976; Henry 1988). The outermost part of the grain, the husk (lemma and palea), contains almost all the lignin of the grain but hemicellulose and cellulose fibres are also present (Munck 1981). Next is the pericarp (fruit coat), which has a chemical composition that resembles the husk except for the lack of lignin.

The pericarp is closely attached to the testa, which surrounds the endosperm. The endosperm is the dominant tissue in grains and also the portion with the most value. It consists of an aleurone layer and starchy endosperm. The aleurone layer of barley is two or three cells thick, while for wheat, oats and rye is only one cell thick, and constitutes about 5% of the grain (Fincher and Stone 1986). Aleurone cells are block-like, with thick walls and large nuclei occurring in layers surrounding the starchy endosperm (Henry 1988). The aleurone cell walls are built mainly of arabinoxylan and β-glucan (Bacic and Stone 1981). In barley, the aleurone cell walls contain about 67% arabinoxylan and 26% β-glucan, in contrast with the starchy endosperm cell walls of barley, which contain mainly β-glucan (75%) and only 20% arabinoxylan (Fincher and Stone 1986). The starchy endosperm cell walls enclose starch granules embedded in a protein matrix. The germ, which consists of scutellum and embryo, comprises about 3% of the grain and is rich in protein and fat. The variations between them and their advantages during growth and development.

Overall composition of barley grain varies from one cultivar to another. Barley cultivars have been classified according to their amylase content in starch as high-amylose, normal and waxy types. CDC Candle variety is a waxy type barley (low amylose) with a higher β-glucan content. Although grown in small quantities, in western Canada, the typical waxy types of barley grown are CDC Candle, CDC Fibar, CDC Alamo, and CDC McGwire. In terms of the end uses, barley cultivars are also classifies as malt, feed and food barley. In general, those with a high β-glucan content are classified as food barley whereas malt barleys have been bred to have a low β-glucan content since

the presence of β-glucan leads to filtration problems during the brewing process. The majority of the barley grown in western Canada is comprised of malt and feed barleys.

2.5. Non-starch polysaccharides in cereals

Non-starch polysaccharides (NSP) were isolated for the first time in 1927 from wheat flours. Their main structure was composed of pentoses, arabinose and xylose (Hoffman 1991) (Fig. 2.7). This combination of polysaccharides was also found in other cereals, examples of which are durum wheat, rye and barley (Table 2.3).

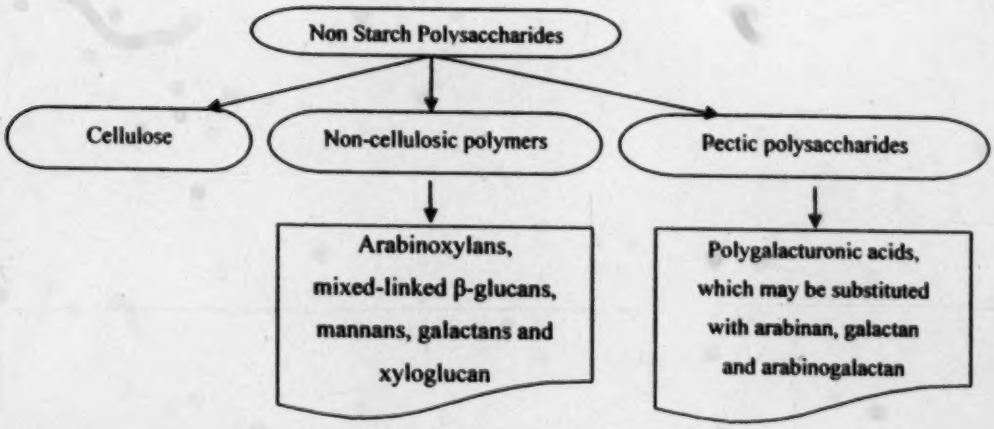
Non Starch Polysaccharides
Cellulose
Non-cellulosic polymers
Pectic polysaccharides
Arabinoxylans, mixed-linked β-glucans, mannans, galactans and xyloglucan
Polygalacturonic acids, which may be substituted with arabinan, galactan and arabinogalactan

Figure 2.7. Division of Non-Starch Polysaccharides and their possible linkages (Adapted from Choct 1997)

Table 2.3. The types and levels of NSP present in some cereal grains and (% w/w, d.b)[1]

Cereal	Arabino-xylan	β-Glucan	Cellulose	Mannose	Galactose	Uronic Acid	Total
Wheat							
Water soluble	1.8	0.4	-	T	0.2	T	2.4
Water insoluble	6.3	0.4	2	T	0.1	0.2	9
Barley							
Water soluble	0.8	3.6		T	0.1	T	4.5
Water insoluble	7.1	0.7	3.9	0.2	0.1	0.2	12.2
Rye							
Water soluble	3.4	0.9	-	0.1	0.1	0.1	4.6
Water insoluble	5.5	1.1	1.5	0.2	0.2	0.1	8.6

[1] Adapted from Englyst (1989).
T: Traces

2.5.1. β-Glucan

The cell wall is a reinforced and multi-component matrix. Although the polysaccharides are their principal constituents, other components (mainly structural proteins) have the ability to form secondary networks inside this matrix (Bacic and Stone 1981). It is well known that major constituents of some portions of the cereal cell wall are arabinoxylans and (1-3)(1-4)-β-glucan. The matrix of arabinoxylans and (1-3)(1-4)-β-glucan in the starchy endosperm represents close to 95% of the cell walls by weight, thus the secondary fibrillar network and cellulosic portion constitute a small portion of the wall.

In barley, the (1-3)(1-4)-β-glucan consists of linear chains of β-glucosyl residues polymerized through both (1-3) and (1-4) linkages. The β-glucan is formed by a group of heterogeneous polysaccharides with respect to size, solubility and the molecular structure (Bacic and Stone 1981; Woodward et al 1983; Woodward et al 1988).

Barley β-glucan has been studied for many years due to its well known human health benefits especially in reducing the risk of cardiovascular heart diseases. At present, the U.S. Food and Drug Administration (FDA), has authorized a health claim for β-glucan from oats recognizing that it may reduce the risk of heart disease (Knuckles et al 1997). These developments led to a growing interest in fractionation of barley and oats in an effort to isolate β-glucan and utilize it as a functional food ingredient.

Some examples of β-glucan isolation have been reported with the objective of facilitating barley fractionation and recovery of β-glucan. Bhatty (1995) isolated β-glucan from pearling flour at lab and pilot plant scale. β-Glucan was extracted using an alkali solution (NaOH) for 1 hr at room temperature, followed by centrifugation (6,500 x g) to separate the soluble and insoluble fractions, adjustment of pH to 6.5 for enzymatic incubation of the soluble fraction with amylase, and precipitation of soluble fibre with ethanol to finally be separated and freeze dried. Temelli (1997) extracted β-glucan from whole barley flour using aqueous alkali ($NaHCO_3$) at different pH (7-10) and temperatures (40-55°C) for 30 min, followed by centrifugation (15,000 x g), precipitation of the soluble fraction with ethanol and air drying.

2.5.2. Arabinoxylans

Arabinoxylans have been found in a variety of tissues of commercial cereals such as wheat, rye, barley, oat, rice and sorghum (Fincher and Stone 1986), as well as other plants, the pangola grass (Ford 1989), bamboo shoots and rye grass (Hartley and Jones 1976). Arabinoxylans are a mixture of polysaccharides composed of 1, 4-linked-β-D-xylose backbone chain and side chains of arabinose attached at the 2 and 3 positions. Arabinoxylans present a similar structure in all cereals. The differences in arabinoxylans from different cereals are believed to be small, but still they are not fully understood. These polysaccharides are considered as minor components of the entire cereal grain, even though they are an important constituent of plant cell walls. The arabinoxylans comprise 60-70% of the aleurone layer and thin cell walls of the starchy endosperm in most cereals (Fincher and Stone 1986).

Arabinoxylans have received interest from cereal chemists and technologists for years, but the structural studies carried out by Perlin (1951a; 1951b) were just taken up in the 1990s because of the growing interest to define the structural characteristics of these polysaccharides. The main structure of arabinoxylans is comprised of pentoses, arabinose and xylose (Fig. 2.8); therefore, they are a component of pentosans, a class of polysaccharides made up of 5-carbon sugars. Cereal arabinoxylans can also contain some hexoses and hexuronic acids as minor constituents. These components are better referred to as heteroxylans (Fincher and Stone 1986).

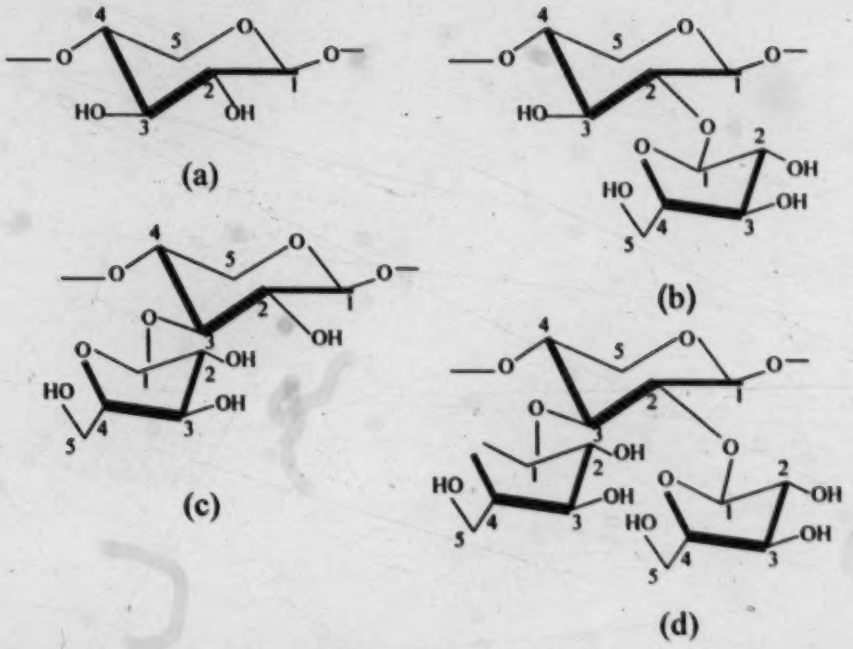

Figure 2.8. Structures of arabinoxylans from barley: a) unsubstituted xylose residue, b) xylose residue substituted at O-2 with arabinose, c) xylose residue substituted at O-3 with arabinose, and d) xylose residue substituted at O-2 and O-3 with arabinose (Adapted from Debyser et al, 1997).

Cereals that contain pentosans should be investigated further, not only because of their pentosan content but also for the uses and importance these cereals have in our lives. For example, wheat is a major part of our diet; a high proportion of our food products is prepared with wheat or contains wheat-based ingredients. Pentosans in wheat have been studied extensively in recent years because of their effects (i.e. water retention, firmness) on the preparation of products like bread. The main advances in wheat pentosans research have been related to water balance, rheological properties, and their impact on starch retrogradation and bread quality (Izydorczyk and Biliaderis 1995). Non-starch

polysaccharides are the main component of wheat bran, and these include arabinoxylans, cellulose and β-glucan, representing between 40-60% (w/w) of wheat bran. Arabinoxylans in wheat represent 70% of the total NSP (Fincher and Stone 1986). The arabinose:xylose ratio (ara/xyl ratio) is about 0.5 (Izydorczyk and Biliaderis 1995).

Oats contain just 3% of water-soluble arabinoxylans in their bran and their structure was established assuming similar configurations to those of other cereals. Rye bran arabinoxylans are similar in structure to wheat bran arabinoxylans but their content is higher than that of oats (~8%).

Arabinoxylans present a significant difference in barley cell walls (20%) and rice (40%) (Fincher and Stone 1986). In comparison, the pericarp and testa of wheat have higher arabinoxylans content (64%) (Ring and Selvendran 1980) and a considerable amount of other components like acidic polymers, glucuronoarabinoxylans (60%) (Dupont and Selvendran 1987). These components have also been found in sorghum (Woolard and Novellie 1976), barley (Fincher and Stone 1986), rice bran and endosperm and some cell walls of maize (Shibuya and Iwasaki 1985).

2.5.2.1. Functional properties

The combination of arabinoxylans and other NSP form the cell walls of grains and thus they are an important part of the skeletal frame of tissue integrity (Fincher and Stone 1986). These polysaccharides may aid the transport of dissolved metabolites and nutrients through the pores around the cellulose crystals. It is also believed that some structural features of arabinoxylans and their mixtures with other polysaccharides (β-

glucan and/or cellulose) is based on non-covalent interactions allowing intermolecular alignment between polymer chains and therefore, the formation of multi-component gels in the cell wall. Ferulic acid residues are also present on the arabinoxylan chains creating potential for covalent polysaccharide-polysaccharide or polysaccharide-protein interactions (Fincher and Stone 1986). Viscoelastic properties of arabinoxylans could be altered by differences in molecular features: degree of branching, spatial arrangement of arabinose/xylose substituents, xylan backbone and ferulic acid content.

Considering the specific role of pentosans in different processes and the differences in pentosans, it was found that the wheat pentosans play a role in bread making (Izydorczyk et al 2001). The variations registered between experimental results reported for wheat pentosans have been attributed to diverse factors such as the methodology used to isolate them, purity, composition and type of pentosans and the systems used to bake them. In addition, other factors such as water absorption, dough development times, amount and molecular sizes have also been considered (Izydorczyk and Biliaderis 1995).

Another consideration to understand the behavior of arabinoxylans is their interactions with other grain components such as starch and their effect on starch retrogradation and thus staling of baked products. The positive effects of arabinoxylans have been attributed to their relation with water activity, and improving the quality of the structural matrix (Izydorczyk and Biliaderis 1995).

Additional functional properties of arabinoxylans in bread-making have been associated with their capacity to retain gas in the dough matrix (Izydorczyk et al 2001)

and the protection of proteins during heat treatment. The high viscosity of arabinoxylans is believed to provide special resistance and strength to the gluten-starch matrix, improving extensibility of dough and providing a compact structure during baking (Izydorczyk et al 2001).

Other important properties of arabinoxylans are those related to malting and brewing processes. It is presumed that in the presence of β-glucan, the arabinoxylans could affect beer processing, generating low yields, filtration problems and increased viscosity and haze. However, there is no clear explanation for these effects and the reactions that take place during malting and brewing (Izydorczyk et al 1998). Therefore, more research is needed to have a better understanding of these and other reactions.

2.5.2.2. Health benefits

In recent years, evidence supporting the fact that the use of NSP may have a direct effect on human health has provided an interesting view that could lead to further research. With the actual importance of health in people's lives and the expected beneficial effects of food as an important factor impacting health and quality of life, the food industry is faced with a major challenge: to develop new products with health benefits. These conditions will only be satisfied by an industry that could identify new sources of natural and nutritional materials and at the same time be capable of generating the desirable sensory quality characteristics in the final food products. As mentioned above, barley possesses two major non-starch polysaccharides: β-glucan and arabinoxylan. Although these two polysaccharides are considered as minor components,

they comprise approximately 15 to 17% of the barley grain, a significant quantity of the grain that should be considered for future use.

Even though β-glucan presents some problems in brewing and animal feed industries, research over the last decade has demonstrated its health benefits. Consequently, the FDA (Food and Drug Administration) of the U.S., in 1997 has approved a health claim to recognize the effect of oat β-glucan as an active ingredient that could reduce the risk of coronary heart disease (Wood and Beer 1998). As well, other studies on barley β-glucan are under review by FDA with the possibility of being approved in the near future. In addition, other benefits of β-glucan are also under investigation. For example, the negative correlation between glycemic index and β-glucan viscosity was reported by Wood et al (1990).

The importance of arabinoxylans in human health has not been investigated and further research is needed to determine its effects on different conditions and provide the background necessary for it to be considered for future health claims.

2.6. Barley arabinoxylans

Barley arabinoxylans are a minor component of the barley endosperm cell walls (Ballance and Manners 1978), but a major element in the aleurone cell walls (Bacic and Stone 1981). Comparatively, barley arabinoxylans contain similar linkages to those found in wheat arabinoxylans; the only difference is the two substituted xylose linkages found in the arabinoxylans of the barley aleurone layer. The whole barley grain contains 6.6% while the endosperm has only 1.4% (w/w) arabinoxylans (Henry 1987). It has been

shown that 46% of the total grain arabinoxylans are located in the husk, 6% in the pericarp, 24% in the aleurone and sub-aleurone layers and 24% is found in the endosperm (McNeil et al 1975). All the polysaccharides in the barley aleurone layer possess 85% pentoses (McNeil et al 1975).

The barley arabinoxylans, like the β-glucan, are a family of polysaccharides where the molecular size, monosaccharide composition, structure and solubility differ individually (Victor et al 1992). Structurally, these arabinoxylans have a (1-4)-β-xylofuranosyl backbone to which α-L-arabinofuranosyl residues are attached through C(O)3 and C(O)2 of the xylosyl residues; however, some other minor residues could also be present (Ballance and Manners 1978; Bacic and Stone 1981; Ballance et al 1986). It has been reported that arabinoxylans are linked to phenolic acids, specifically to ferulic and p-coumaric acids linked via an ester linkage to C(O)-5 of the arabinose residue (Ballance 1986). These acids show an important covalent association with arabinoxylans and represent about 0.05% of cell walls in the starchy endosperm (Victor et al 1992) and 1.2% of the aleurone cell walls (Bacic and Stone 1981). Ferulic acid has the ability to form ester and ether linkages, and it is assumed that it can participate in the cross-linking of macromolecules in the cell wall, thus making them less susceptible to digestion. However, the true association between ferulic acids and their functionality in arabinoxylans has not been fully explained (Fig. 2.9) and more research is needed.

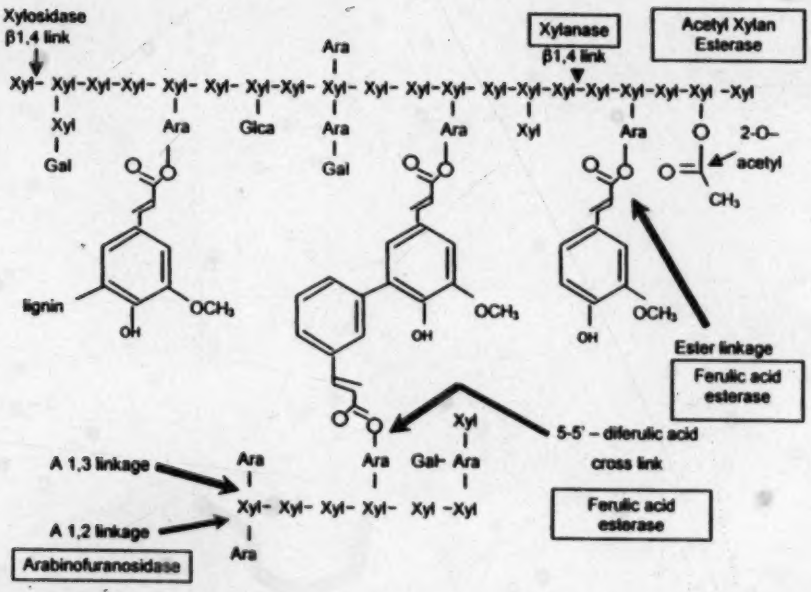

Figure 2.9. Structure of barley arabinoxylans and the presence of ferulic acid linkages (Adapted from Rasmusson, 1985)

2.6.1. Physicochemical properties

Arabinoxylans in barley seem to be more structurally uniform than those in wheat. Studies on the water-insoluble barley arabinoxylans, hydrolyzed with endoxylanase from *Aspergillus awamori*, have shown two major substitution patterns for these polysaccharides (Victor et al 1992). Singly or doubly substituted xyloses are joined together but separated by single unsubstituted residues. These groups are interlinked, using as intermediaries short un-branched regions of xylose residues and it is due to the un-branched regions that there are possible differences in the arabinose/xylose ratio of these polysaccharides.

Determination of the molecular weight of cereal arabinoxylans is very complicated and hence, their molecular weight may vary greatly depending on the method used for their determination. For example, water-extractable wheat arabinoxylans have been reported to have a molecular weight range of 65,000 to 66,000 Daltons, determined by sedimentation (Izydorczyk and MacGregor 2000), whereas this range was 800,000 to 5,000,000 Daltons using gel filtration technique (Fincher and Stone 1986). On the other hand, molecular weights of up to 5,000,000 Daltons (MacGregor and Fincher 1993) have been determined by gel filtration chromatography for the barley arabinoxylans.

2.6.2. Extraction

Studies on isolation, fractionation and structural characterization of barley arabinoxylans or pentosans have revealed that a major portion of these polysaccharides is not water-extractable (Izydorczyk et al 1998), but the reason for such insolubility has not been completely revealed. Some speculations indicate the existence of ester linkages binding the insoluble fraction to the starchy endosperm cell walls (Cui and Wood 1999). The difficulty of extracting barley arabinoxylans with aqueous solutions can be related to different acidic bridges around the arabinoxylan chains (Cui 2001) or the presence of non-covalent interactions between β-glucan-cellulose and β-glucan-arabinoxylans. However, the existence of such interactions is not fully demonstrated. In addition, arabinoxylans can be separated using different methodologies under different conditions. They are mainly not soluble in water but alkaline solvents can be used to extract them

(i.e. NaOH and $Ba(OH)_2$). Water-soluble arabinoxylans in cereals have been successfully separated from residues (insoluble fraction) using aqueous preparations (Fincher and Stone 1986; Gruppen et al 1989).

The main challenge for these separations is the purification of arabinoxylans from contaminating water-soluble components (proteins, α- and β-glucans, and arabinogalactans).

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3. QUANTIFICATION OF PENTOSANS IN SOLUBLE AND INSOLUBLE FRACTIONS OF BARLEY PEARLING FLOUR[1]

3.1. Introduction

Barley is one of the major cereals with great potential in the food industry due to its composition. Barley represents an interesting option for future food product applications as its components located in the outer layers could provide human health benefits. Increased human consumption of such products will also lead to increased fibre intake to meet the recommendations of nutritionists. In recent years, the growing interest for understanding the possible interactions between Non-Starch Polysaccharides (NSP), specifically, β-glucan and pentosans and other major and minor components of barley grain has led to increased research and development of new methodologies for extraction and purification of these fibre components.

As in other grains, component interactions in the pearling flour are not fully explained. Strong interactions between the components in the matrix making up the pearling flour impact the ease of isolation of various components. To accomplish the extraction of barley pentosans, different methods should be developed or adopted, based on past research. The extraction and isolation of pentosans have been focused mainly on wheat due to the importance of wheat in many staple food products. For example, Gruppen et al (1990) established a method for the isolation of water-insoluble cell wall material from wheat flour (dough form) and their application to large scale processing.

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[1] A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

Similarly, Faurot et al (1995) developed a method for the isolation of water-soluble and insoluble pentosans from wheat flour to be used in large scale production. Bataillon et al (1998) established extraction and purification of arabinoxylans from previously destarched wheat bran on a pilot scale. Delcour et al (1999) established a method applicable to large scale processing to isolate water-extractable arabinoxylans from rye due to their high pentosan content.

Research on other cereals containing pentosans, especially barley has been very limited. Therefore, more research is needed to better understand the solubility behavior and extractability of barley pentosans. Barley arabinoxylans are one of the major NSP present in cereal aleurone cell walls. They are water-insoluble polysaccharides (Cui and Wood 1999), but are soluble in alkali solutions and their extractability was proportional to NaOH concentrations (Victor et al 1994). Izydorczyk (1998) presented the structure and physicochemical properties of barley NSP, and their extraction using a combination of aqueous extraction and enzymatic treatments. Pentosans, located in the outer layers of the pearling flour are attached firmly to proteins, phenolic acids and possibly starch; however, there is not enough research to demonstrate such interactions. Thus, more research is needed on these specific linkages and the effect they have on the solubility of pentosans in order to improve future processing operations targeting the recovery of pentosans. In addition, more information is needed to establish simultaneous distributions of pentosans, β-glucan and proteins in the soluble and insoluble fractions to understand how components distribute during fractionation processes.

The overall objective of this study was to enhance our understanding of the distribution of soluble and insoluble pentosans under specific conditions of temperature, pH and alkali concentrations and test the hypothesis that barley pentosans are mainly insoluble. The specific objectives were:

1. to perform extractions of barley pearling flour under different conditions of temperature, pH and initial alkali concentration,
2. to analyze the composition (pentosans, β-glucan and proteins) of soluble and insoluble fractions obtained, and
3. to evaluate the color of soluble and insoluble fractions obtained to elucidate the impact of phenolic acids.

3.2. Materials and Methods

3.2.1. Materials

CDC Candle barley grains, a hull-less waxy barley variety, were provided by Agricore United (Calgary, AB). The 19% Pearling Flour (PF) fraction obtained by Lekhi (2003) was previously stored at 4°C and used as the starting material in this study. Prior to use in the extraction experiments, any clumps present in the pearling flour sample were manually broken on a 0.5 mm screen to ensure that the whole sample passed through. Sodium hydroxide, 98.5% of purity, ethanol 100% purity, and HCl, 37.5% purity, were obtained from Sigma Chemical Co. (St. Louis, MO).

3.2.2. Extraction procedure

The extraction protocols used are presented in Figures 3.1 and 3.2. Three sets of extractions were carried out. The first and second sets were performed under similar pH but different initial alkali concentrations according to Figure 3.1. The third set was performed at different temperature conditions using water or NaOH as solvent according to Figure 3.2. Barley pearling flour samples (20 and 40 g) were dispersed in 1:25 (w/v) volumes of appropriate solvent at different pH levels, pH was re-adjusted to the desired level and maintained for the entire extraction period of 1 hr with constant stirring in a water bath at different temperatures.

Solutions with initial concentrations of 0.04M, 0.07M, 0.1M 0.15M, 0.2M NaOH with pH adjustments to 9 and 11 using HCl and an extraction temperature of 55°C were the conditions used for set 1. Solutions with initial concentrations of 0.1M, 1M, 2.5M NaOH with pH adjustments to 9 and 11 using HCl and extraction temperature 55°C were followed for set 2. Solution with initial concentration of 0.1M NaOH and water, with pH adjustment to 6 using HCl and an extraction temperatures of 23°C (ambient conditions), 55°C (chosen to minimize starch gelatinization and thus minimize dilution of pentosans in the soluble fraction) and 90°C (boiling condition) were used for set 3.

In all cases, the flour slurry was centrifuged (Model J2-21 Beckman Instrument Co., Mississauga, ON) after the extraction period for 15 min at 18.000 x g at 15°C to remove insoluble solids.

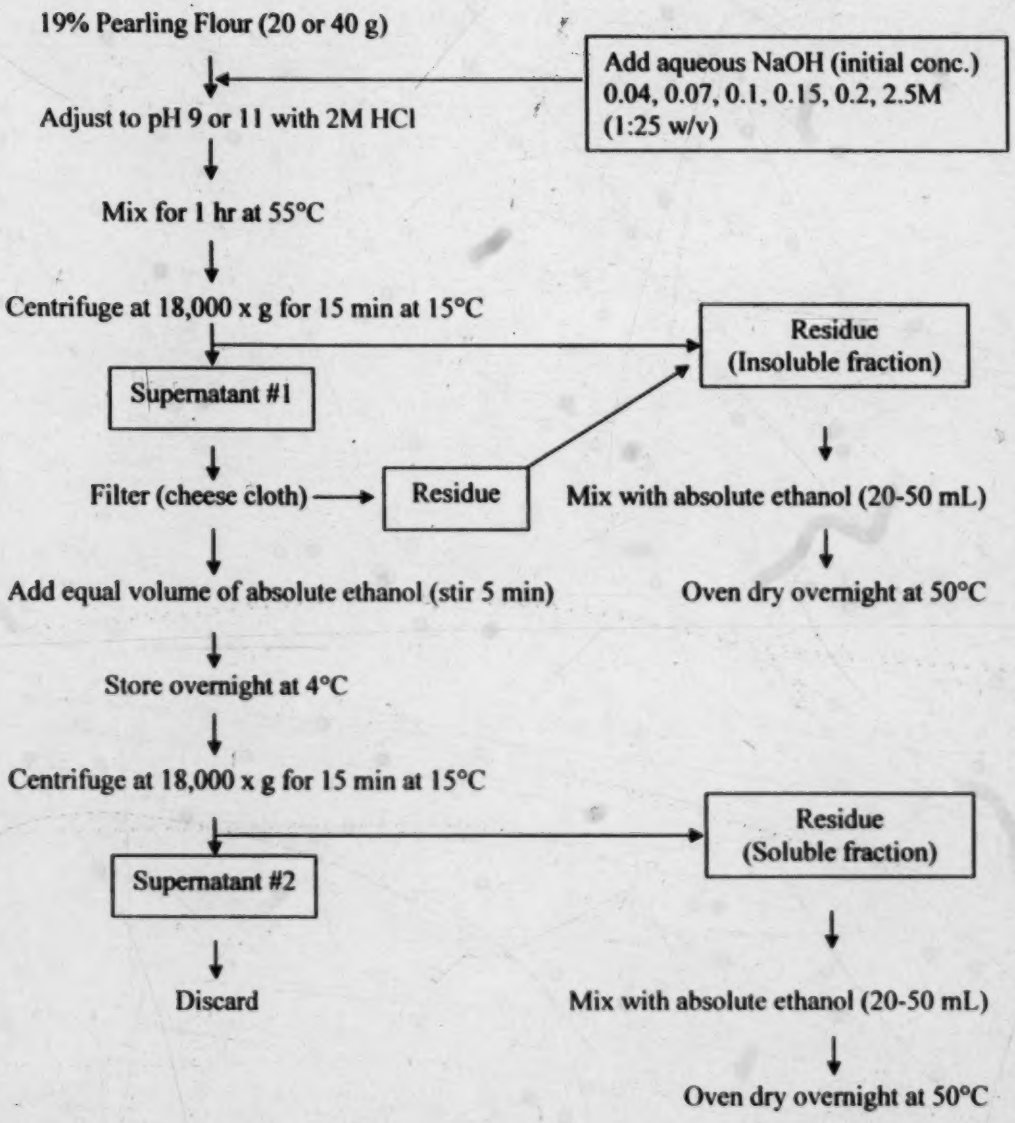
19% Pearling Flour (20 or 40 g)
Add aqueous NaOH (initial conc.) 0.04, 0.07, 0.1, 0.15, 0.2, 2.5M (1:25 w/v)
Adjust to pH 9 or 11 with 2M HCl
Mix for 1 hr at 55°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Insoluble fraction)
Supernatant #1
Filter (cheese cloth)
Residue
Mix with absolute ethanol (20-50 mL)
Add equal volume of absolute ethanol (stir 5 min)
Oven dry overnight at 50°C
Store overnight at 4°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Soluble fraction)
Supernatant #2
Discard
Mix with absolute ethanol (20-50 mL)
Oven dry overnight at 50°C

Figure 3.1. Flow diagram for the recovery of soluble and insoluble fractions from 19% pearling flour of CDC Candle barley at different initial alkali concentrations and pH.

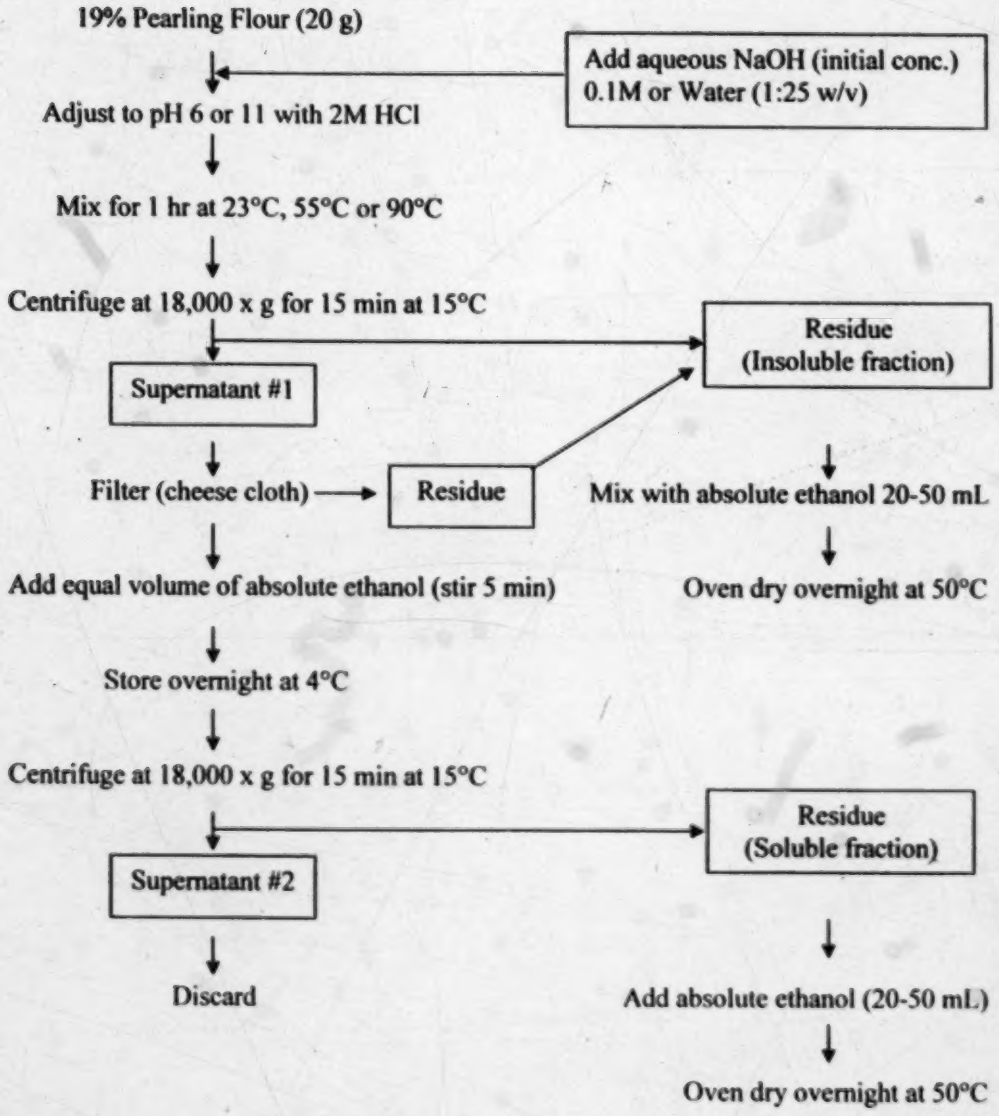
19% Pearling Flour (20 g)
Add aqueous NaOH (initial conc.) 0.1M or Water (1:25 w/v)
Adjust to pH 6 or 11 with 2M HCl
Mix for 1 hr at 23°C, 55°C or 90°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Insoluble fraction)
Supernatant #1
Filter (cheese cloth)
Residue
Mix with absolute ethanol 20-50 mL
Add equal volume of absolute ethanol (stir 5 min)
Oven dry overnight at 50°C
Store overnight at 4°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Soluble fraction)
Supernatant #2
Discard
Add absolute ethanol (20-50 mL)
Oven dry overnight at 50°C

Figure 3.2. Flow diagram for the recovery of soluble and insoluble fractions from 19% pearling flour of CDC Candle barley at different temperatures.

The insoluble fraction was recovered, mixed with ~50 mL of absolute ethanol and dried in an isothermal oven (Model 655G, Fisher Scientific, Edmonton, AB) at 55°C for 20 hr (overnight).

The supernatant, obtained in the first centrifugation step, was decanted and filtered through a cheese cloth (with 100-200μm opening). The portion of the insoluble fraction recovered in this step was combined with the residue from the first centrifugation step prior to ethanol addition and drying. An equal volume of absolute ethanol was added to the supernatant to precipitate the soluble fraction. Ethanol was added with constant stirring for 5 min to obtain a 50% (v/v) ethanolic solution, which was left undisturbed overnight at 4°C to precipitate the NSP (pentosans and β-glucan). The next day, the NSP were recovered by centrifugation at 18,000 x g at 15°C. The supernatant resulting from this stage was discarded and the residue (soluble fraction) was mixed with ~50 mL of absolute ethanol and left in the fume hood for 15 min. The residue (soluble fraction) was then dried in an isothermal oven at 50°C for 20 hr (overnight).

The dried samples (soluble and insoluble fractions) were ground first in a coffee grinder (Model KSM2(4) Braun, DF, Mexico) for 2 min and then manually in a mortar and pestle until the particle size was small enough to pass through a 0.5 mm screen. Samples were collected, and stored at room temperature for compositional analysis. Analyses were performed within the next 2 days following the extraction.

3.2.3. Composition analysis

Pentosan content determination was performed following the orcinol and HCl method of Hashimoto et al (1987) after some modifications. These modifications consisted of increased dilution of the sample at the final step from 1 mL to 18 mL and substitution of the filtration step with microfugation (speed 10, 5 min). This method of pentosan content determination is based on acid digestion of the sample followed by neutralization, fermentation of hexoses with fresh baker's yeast and color development by pentoses, with orcinol and ferric chloride which was determined spectrophotometrically. The β-glucan determination was done according to McCleary and Glennie-Holmes (1985) using the Megazyme assay kit (Megazyme Inc., Wicklow, Ireland). Protein content was determined using a Nitrogen analyzer (Model FP 428, Leco Corp., St. Joseph, MI) (%N x 6.25). Moisture content was determined using the AACC method 44-19 (AACC, 1982). All analyses were performed in duplicate.

Percentage of pentosans, β-glucan or protein not solubilized and the recovery of each of these components were calculated and reported. For example, for pentosans: Percentage of pentosans not solubilized = (g pentosans in insoluble fraction)*100/(g pentosans in starting material).

Recovery of total pentosans = (g pentosans in soluble fraction + g pentosans in insoluble fraction)*100/(g pentosans in starting material)

3.2.4. Color determination

Color measurements of the soluble and insoluble fractions obtained from 19% PF Candle barley were performed in duplicate using a LabScan XE Hunterlab color difference meter (Model D52-2, Hunter Associates Laboratory, Fairfax, VA). Color values for Hunter *L* (0 black, 100 white), *a* (⁻green, ⁺red) and *b* (⁻blue, ⁺yellow) values were recorded. Calibration of the instrument was performed using a white tile with known values of *L* (91.66), *a* (-1.00) and *b* (-0.20).

3.2.5. Statistical analysis

The extraction protocols at each combination of pH/solvent/alkali concentration/temperature were carried out in duplicate to obtain soluble and insoluble fractions of CDC Candle pearling flour. The compositional analysis of each fraction obtained was also performed in duplicate. Analysis of variance for the results of each set of extraction was done using the General Linear Model (GLM) procedure of SAS Statistical Software, Version 8.2 (SAS Institute, 2002). The model for the first two sets of data consisted of the main effects of pH and alkali concentration and their interaction effects on pentosan, β-glucan and protein contents. The model for the third set of data consisted of the main effects of pH, type of solvent, temperature and their interaction effects. In all cases, multiple comparison of means was performed by Tukey's studentized HSD (Honestly Significant Difference) ranged test at $\alpha = 0.05$ level of confidence.

3.3. Results and discussion

CDC Candle barley variety was selected for this study as a representative of the food barley class. Pearling flour was chosen as the starting material for pentosan extraction since pentosans are concentrated more in the outer layers of the barley grain. As part of a continuation of a previous work performed by Lekhi (2003), CDC Candle barley 19% PF was analyzed, showing the following compositional results as presented in Table 3.1: The moisture content for the samples ranged from 8 to 14%. Pentosan content was 11.14%, 4.86% of β-glucan and 24.81% of protein. The starch and lipid contents were 18.64% and 9.55%, respectively. Pentosan, β-glucan and protein contents were determined on the extracts obtained at different conditions and the results are presented in the following sections.

Table 3.1. Composition of CDC Candle 19% pearling flour[1]

Component	% (w/w d.b)
Starch	18.64
β-Glucan	4.86
Pentosans	11.14[2]
Lipids	9.55
Protein	24.81
Ash	6.00

[1]Adapted from Lekhi (2003)
[2]Determined in this study

3.3.1. Composition and yields of fractions (soluble and insoluble) as a function of solvent, pH and temperature

3.3.1.1. Pentosans

The combinations of pH and initial alkali concentrations led to interesting results about the distribution of soluble and insoluble pentosan fractions and are presented in Tables 3.2-3.4. Three sets of extractions were performed. In the first set of extraction, carried out at pH 9, 11 and initial alkali concentrations of 0.04-0.2M, both pH and alkali concentration had a significant effect ($p \leq 0.05$) on the pentosan content of the soluble and insoluble fractions. The insoluble fractions had a higher pentosan content compared to those obtained in the soluble fractions, presenting a range from 16 to 22% and 5 to 11%, respectively (Table 3.2).

It is important to note that there was a substantial loss in the amount of sample recovered compared to the 20 g starting material since the total recovery was only approximately 14 g. The loss was associated with the aqueous ethanol portion discarded, following precipitation of the soluble fraction. The loss of sample was confirmed after drying a portion of the ethanolic residue to recover the solids, which resulted in the recovery of 1 g per 100 mL of ethanolic residue (total amount in a typical run was ~700 mL). This finding demonstrated that the use of 1:1 (v/v) ethanol addition was not effective for the recovery of all solubles.

Table 3.2. Pentosan content of soluble and insoluble fractions of CDC Candle pearling flour (20 g) obtained with NaOH at different pH and initial alkali concentration levels at 55°C[1]

pH	Initial NaOH Concentration (M)	Soluble fraction		Insoluble fraction		% of pentosans not solubilized	Recovery of total pentosan (%)
		Weight (g)	Pentosan (% w/w, d.b)	Weight (g)	Pentosan (% w/w, d.b)		
9	0.04	1.58	5.41^{c}	10.35	18.42^{ab}	85.63	82.96
9	0.07	1.76	8.07^{abc}	10.95	17.38^{ab}	85.42	85.88
9	0.10	2.17	6.53^{abc}	11.21	15.02^{b}	75.69	74.85
9	0.15	2.52	7.36^{abc}	11.36	16.30^{ab}	79.31	77.52
9	0.20	5.12	5.49^{c}	9.07	17.71^{ab}	71.39	77.23
11	0.04	1.40	10.72^{ab}	9.43	22.56^{a}	95.43	95.07
11	0.07	1.64	10.97^{a}	8.94	20.44^{ab}	82.16	83.49
11	0.10	1.54	8.53^{abc}	9.98	18.01^{ab}	80.82	80.39
11	0.15	2.04	9.03^{abc}	9.34	17.68^{ab}	74.15	75.41
11	0.20	3.99	6.28^{bc}	8.28	17.97^{ab}	66.69	71.35

[1]All extractions and analysis of each sample were performed in duplicate.

$^{a-c}$Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.3. Pentosan content of soluble and insoluble fractions of CDC Candle pearling flour (40 g) obtained with NaOH at different pH and initial alkali concentration levels at 55°C[1]

pH	Initial NaOH Concentration (M)	Soluble fraction		Insoluble fraction		% of pentosans not solubilized	Recovery of total pentosans (%)
		Weight (g)	Pentosan (% w/w, d.b)	Weight (g)	Pentosan (% w/w, d.b)		
9	0.2	15.50	9.58^{c}	8.66	18.82^{a}	29.02	59.79
9	1.0	19.72	11.46^{b}	8.29	11.83^{b}	19.43	64.07
9	2.5	11.35	7.77^{d}	13.10	6.36^{d}	21.99	30.90
11	0.2	6.77	16.14^{a}	8.84	10.24^{c}	39.45	35.41
11	1.0	10.30	11.14^{b}	18.73	5.97^{d}	25.73	41.93
11	2.5	11.05	7.35^{d}	13.58	6.31^{d}	40.86	28.83

[1]All extractions and analysis of each sample were performed in duplicate.
[a-d]Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.4. Pentosans content of soluble and insoluble fractions of CDC Candle pearling flour (20 g) obtained with 0.1M NaOH (initial concentration) and water at different temperatures and pH.[1]

pH	Solvent	Temp (°C)	Soluble fraction		Insoluble fraction		% of pentosans not solubilized	Recovery of total pentosan (%)
			Weight (g)	Pentosan (% w/w, d.b)	Weight (g)	Pentosan (% w/w, d.b)		
6	water	23	4.06	8.30[a]	13.26	10.48[b]	62.38	67.66
6	water	55	4.21	8.91[a]	12.86	9.97[b]	57.55	64.35
6	water	90	3.98	5.88[a]	13.09	12.99[b]	76.37	78.37
11	NaOH	23	9.42	3.71[b]	8.77	12.70[b]	50.01	54.44
11	NaOH	55	7.10	5.39[a]	10.10	17.65[a]	80.01	86.49
11	NaOH	90	10.95	7.90[a]	9.44	18.17[a]	49.89	55.05

[1]All extractions and analysis of each sample were performed in duplicate.
[a,b]Means with different letters within a column are significantly different ($p \leq 0.05$).

The distribution of pentosans between the soluble and insoluble fractions was evaluated by determining the portion of the pentosans remaining in the insoluble residue after extraction under different conditions as a percentage of the total pentosans in the feed material. It was found that 66-95% of pentosans remained in the insoluble fraction. Under these set of pH/alkali concentration conditions and constant temperature, the recovery of total pentosans ranged from 71-95% (Table 3.2), pointing to the losses in the discarded aqueous ethanol portion.

The second set of extractions (Table 3.3) was also performed under similar conditions used for set one, but at higher initial alkali concentrations (0.2M, 1M, 2.5M). Both pH and alkali concentration had a significant effect ($p \leq 0.05$) on the pentosans content of the soluble and insoluble fractions. Significantly higher ($p \leq 0.05$) pentosan content was achieved at pH 11/0.2M NaOH in the soluble fraction (16.14%) and pH 9/0.2M NaOH in the insoluble fraction (18.82%).

The percentage of pentosans not solubilized was 19-41% and the total recovery was 28-64%. These values were substantially lower than those reported in Table 3.2, indicating the higher extent of solubilization of pentosans, but they were not recovered by ethanol precipitation.

The third set of extractions was performed under different temperature (23, 55, 90°C) and pH conditions (pH 6, 11) and the use of water and 0.1M NaOH (initial concentration) as the solvent (Table 3.4). The pH, solvent and temperature had a significant effect ($p \leq 0.05$) on the pentosan content of the soluble and insoluble fractions. The pentosan contents of the insoluble fractions obtained at 55°C (17.65%) and 90°C

(18.17%) at pH 11 were similar but significantly ($p \leq 0.05$) higher than those obtained at other conditions. Pentosan contents of the soluble fractions were similar ($p \leq 0.05$), except for that obtained at 23°C/pH 11 using NaOH. The percentage of pentosans remaining in the insoluble fraction was 49-80%, whereas the recovery of total pentosans for these samples was considerable, presenting a range of 50 to 86% (Table 3.4).

Delcour et al (1999), using larger scale isolation of water extractable arabinoxylans from rye flour (wholemeal) with longer extraction periods (90-150 min), similar temperatures (40-90°C) and enzymatic treatments (amylases) obtained 54.2% arabinoxylans as total recovery of arabinoxylans in the soluble and insoluble fractions. Bataillon et al (1998) following a procedure to extract and purify arabinoxylans from destarched wheat bran with NaOH (60-80% concentration) and temperatures of 20, 40, 60, and 80°C presented similar results. Under laboratory conditions, yields of 34.1% and purity of 42.3% were achieved in the product extracted.

However, the results generated in these procedures were not favorable in terms of further application of the extraction method in an industrial or semi-industrial setting due to its high energy costs and the potential for environmental and equipment damages (corrosion).

3.3.1.2. β-Glucan

For β-glucan (Tables 3.5-3.7), the conditions used in the three sets of extractions resulted in differences in the contents of soluble and insoluble fractions, and as expected, in their distribution and recoveries.

Table 3.5. β-Glucan content of soluble and insoluble fractions of CDC Candle pearling flour (20 g) obtained with NaOH at different pH and initial alkali concentration levels at 55°C[1]

pH	Initial NaOH Concentration (M)	Soluble fraction		Insoluble fraction		% of β-glucan not solubilized	Recovery of total β-glucan (%)
		Weight (g)	β-glucan (% w/w, d.b)	Weight (g)	β-glucan (% w/w, d.b)		
9	0.04	1.58	23.64[a]	10.35	2.64[a]	22.67	47.10
9	0.07	1.76	22.40[a]	10.95	2.44[a]	22.26	48.60
9	0.10	2.17	19.56[b]	11.21	2.25[a]	20.99	48.28
9	0.15	2.52	18.81[b]	11.36	2.01[a]	19.48	51.12
9	0.20	5.12	8.43[c]	9.07	2.27[a]	17.06	46.40
11	0.04	1.40	27.48[a]	9.43	2.28[a]	17.85	43.34
11	0.07	1.64	24.41[a]	8.94	2.47[a]	18.33	45.20
11	0.10	1.54	25.00[a]	9.98	1.94[a]	16.14	41.40
11	0.15	2.04	19.90[b]	9.34	2.48[a]	19.23	46.39
11	0.20	3.99	9.99[c]	8.28	2.56[a]	17.59	44.37

[1]All extractions and analysis of each sample were performed in duplicate.

[a-c]Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.6. β-Glucan content of soluble and insoluble fractions of CDC Candle pearling flour (40 g) obtained with NaOH at different pH and initial alkali concentration levels at 55°C[1]

pH	Initial NaOH Concentration (M)	Soluble fraction		Insoluble fraction		% of β-glucan not solubilized	Recovery of total β -glucan (%)
		Weight (g)	β-glucan (% w/w, d.b)	Weight (g)	β-glucan (% w/w, d.b)		
9	0.2	15.50	7.07^{ab}	8.66	2.02^{b}	12.13	50.89
9	1.0	19.72	10.94^{a}	8.29	0.96^{c}	5.40	54.34
9	2.5	11.35	8.39^{ab}	13.10	0.69^{c}	3.40	41.81
11	0.2	6.77	6.03^{b}	8.84	3.44^{a}	7.40	27.17
11	1.0	10.30	6.04^{b}	18.73	1.59^{b}	7.40	54.24
11	2.5	11.05	8.16^{ab}	13.58	0.62^{c}	3.80	41.68

[1]All extractions and analysis of each sample were performed in duplicate.

$^{a-c}$Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.7. β-Glucan content of soluble and insoluble fractions of CDC Candle pearling flour (20 g) obtained with 0.1M NaOH (initial concentration) and water at different temperatures and pH[1]

pH	Solvent	Temp (°C)	Soluble fraction		Insoluble fraction		% of β-glucan not solubilized	Recovery of total β-glucan (%)
			Weight (g)	β-glucan (% w/w, d.b)	Weight (g)	β-glucan (% w/w, d.b)		
6	water	23	4.06	8.26[c]	13.26	2.40[b]	26.90	48.30
6	water	55	4.21	8.95[c]	12.86	2.00[b]	21.80	46.60
6	water	90	3.98	9.99[b]	13.09	1.90[b]	20.50	47.12
11	NaOH	23	9.42	10.30[b]	8.77	1.60[b]	11.60	85.71
11	NaOH	55	7.10	11.84[a]	10.10	2.80[b]	23.10	81.52
11	NaOH	90	10.95	9.86[b]	2.44	4.00[a]	8.10	91.36

[1]All extractions and analysis of each sample were performed in duplicate.

[a-c]Means with different letters within a column are significantly different ($p \leq 0.05$).

In the first set of extractions (Table 3.5), both pH and initial alkali concentration had a significant effect ($p \leq 0.05$) on the β-glucan content of the soluble fraction. The response in β-glucan content was similar to that of pentosan content presented above, showing the effectiveness of lower alkali concentration for their extraction within the range of 0.04 to 0.20M. For the insoluble fractions, there was no significant change ($p > 0.05$) in their β-glucan content due to a change in pH or initial alkali concentration, reaching low values (1.94-2.64%) compared to 4.86% in the starting material. As expected, the β-glucan content was higher in the soluble fraction with a change in pH or alkali concentration, which was reflected in the 16.14-22.67% of β-glucan being not solubilized. The total recovery of β-glucan from the starting material was approximately 45% (Table 3.5), again pointing to the losses in the ethanol stream.

Temelli (1997) performed a study with whole Condor barley flour to extract and characterize the properties of barley β-glucan under different temperature (40°C, 45°C, 50°C, 55°C) and pH conditions (7, 8, 9, 10). Her results do not agree with the results obtained in this study since she achieved a higher β-glucan content (89.01%) in the extracts obtained at 55°C and pH 7 with a constant decrease as pH was increased to 10, resulting in a lower β-glucan recovery (~57%). These results, confirm the poor concentration and β-glucan recoveries obtained in this study (<50%) using pearling flour as the starting material as opposed to the whole grain flour used by Temelli (1997).

For the second set of extractions (Table 3.6) where the initial alkali concentrations was increased from 0.2M to 2.5M NaOH at pH 9 or 11, the β-glucan content of the insoluble fraction showed, as expected, a significant decrease ($p \leq 0.05$) due to the increase in the alkali concentrations. The β-glucan content of the insoluble fraction

obtained using 2.5M NaOH at pH 9 (0.69%) and 11 (0.62%) was significantly ($p \leq 0.05$) lower compared to those obtained at lower alkali concentrations. For the soluble fractions, the β-glucan content was higher than that of the insoluble fraction. The percentage of β-glucan not solubilized (3.40-12.13%) was substantially lower than that obtained at lower alkali concentrations (Table 3.5), indicating the importance of alkali concentration for the solubilization of NSP. The recovery percentages for all conditions ranged from 27.17 to 54.34% (Table 3.6).

The third set of extractions (Table 3.7) resulted in a significant increase ($p \leq 0.05$) in the β-glucan content of both soluble and insoluble fractions when 0.1M NaOH was used at pH 11 at 55°C (11.84%) and 90°C (4.00%), respectively. The highest β-glucan content was found in the soluble fraction at 55°C, pH 11 and 0.1M NaOH (11.84%). This was reflected in the substantially higher total recoveries when the solvent used was 0.1M NaOH (81-91%) compared to water (46-48%). Furthermore, in general, variations in temperature from 23 to 90°C did not have a significant effect ($p>0.05$) on the β-glucan contents of the soluble and insoluble fractions (Table 3.7).

Burkus and Temelli (1998) performed a study to understand the effect of different extraction conditions (55°C and pH 8-10) on the yield and composition of Condor and waxy barley β-glucan gum. They reported lower β-glucan contents (~40-58%) in the extract obtained at higher pH conditions (9/10) in Condor and these levels prevailed low when a wide pH range was used (5-10) in the waxy cultivar. This confirms again, the significant drop in the β-glucan content of the extract when higher pH conditions are used

as well as the importance of barley variety used and whether the starting material is pearling flour or endosperm flour.

3.3.1.3. Protein

In the first set (Table 3.8), both pH and initial alkali concentration had a significant effect ($p \leq 0.05$) on the protein contents of the soluble and insoluble fractions. The higher protein contents were registered in the soluble fraction at initial alkali concentrations of 0.04M (21.12%), 0.1M (20.50%) and 0.15M (19.40%) at pH 9. When the alkali concentration was increased at both pH 9 and 11 there was a significant reduction ($p \leq 0.05$) in the protein content of the soluble fraction.

The results for the percentage of protein not solubilized confirmed the impact that pH had for soluble and insoluble fractions. When pH 9 was used the values ranged from 38 to 48%, meanwhile at pH 11, the range was from 15 to 28%, showing the higher extent of protein solubilization at higher pH. Besides, the total protein recoveries were acceptable at pH 9 (76.32-90.81%), whereas those at pH 11 were very low (26.06-56.84%), leading to conclude that there was a bigger loss in the discarded fraction, possibly because they were solubilized to a greater extent (Table 3.8).

The second set (Table 3.9) under similar temperature (55°C), and pH conditions (9 or 11) but higher initial alkali concentrations (0.2M, 1M, 2.5M) showed a significant effect ($p \leq 0.05$) of pH and alkali concentration on the protein content of soluble and insoluble fractions.

Table 3.8. Protein content of soluble and insoluble fractions of CDC Candle pearling flour (20 g) obtained with NaOH at different pH and initial alkali concentrations levels at 55°C[1]

pH	Initial NaOH Concentration (M)	Soluble fraction		Insoluble fraction		% of protein not solubilized	Recovery of total protein (%)
		Weight (g)	Protein (% w/w, d.b)	Weight (g)	Protein (% w/w, d.b)		
9	0.04	1.58	21.12[a]	10.35	15.17[a]	42.46	76.32
9	0.07	1.76	20.50[a]	10.95	14.13[b]	41.82	77.05
9	0.10	2.17	19.40[a]	11.21	15.73[a]	47.76	88.02
9	0.15	2.52	10.87[c]	11.36	16.18[a]	48.71	84.64
9	0.20	5.12	16.01[b]	9.07	15.95[a]	38.57	90.81
11	0.04	1.40	12.97[c]	9.43	8.74[c]	22.46	36.91
11	0.07	1.64	10.82[c]	8.94	6.45[d]	15.61	26.06
11	0.10	1.54	14.27[b]	9.98	9.60[c]	26.72	45.93
11	0.15	2.04	14.47[b]	9.34	9.08[c]	22.92	42.58
11	0.20	3.99	10.19[c]	8.28	12.65[b]	28.35	56.84

[1] All extractions and analysis of each sample were performed in duplicate.

[a-d] Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.9. Protein content of soluble and insoluble fractions of CDC Candle pearling flour (40 g) obtained with NaOH at different pH and initial alkali concentration levels at 55°C[1].

pH	Initial NaOH Concentration (M)	Soluble fraction		Insoluble fraction		% of protein not solubilized	Recovery of total protein (%)
		Weight (g)	Protein (% w/w, d.b)	Weight (g)	Protein (% w/w, d.b)		
9	0.2	15.50	3.89^{c}	8.66	9.88^{a}	11.48	9.97
9	1.0	19.72	3.78^{c}	8.29	4.33^{b}	4.79	6.99
9	2.5	11.35	2.76^{c}	13.10	2.13^{b}	3.75	0.47
11	0.2	6.77	12.44^{a}	8.84	9.41^{a}	11.17	13.32
11	1.0	10.30	6.98^{b}	18.73	3.90^{b}	9.87	10.70
11	2.5	11.05	4.97^{cb}	13.58	2.45^{b}	4.41	3.73

[1]All extractions and analysis of each sample were performed in duplicate.

[a,b]Means with different letters within a column are significantly different ($p<0.05$).

The highest protein content was achieved in the soluble fraction using 0.2M NaOH at pH 11 (12.44%). The recovery values presented in Table 3.9 are substantially lower compared to those found in Table 3.8 under similar extraction conditions. This is reasonable since the protein content of both fractions (soluble and insoluble) was lower as well as the percentage of proteins not solubilized, leading to the conclusion that at higher alkali conditions more proteins were solubilized but not recovered, resulting in a higher loss.

The third set of extraction results (Table 3.10) showed that the solvent, pH and temperature had a significant effect ($p \leq 0.05$) on the protein content of soluble and insoluble fractions. When water was used at 23°C (ambient temperature), the protein content of the soluble fraction was 20.21%, which was similar to those of fractions obtained with water at 55°C and 0.1M NaOH at 23°C.

When alkali was used (0.1M NaOH) and the temperature was increased to 55°C and 90°C at pH 11, there was a significant decrease ($p \leq 0.05$) in the protein content of the soluble fraction. The protein contents of insoluble fractions obtained with water were similar ($p > 0.05$) but significantly ($p \leq 0.05$) higher than those obtained with NaOH. As expected, the percentage of protein not solubilized with water as the solvent (~44%) was approximately twice as high as that with NaOH (~24%). The total amount of protein recovered showed minimal variations when water (~50%) or 0.1M NaOH (~45%) was used (Table 3.10). However, these values were lower than those presented by Lekhi (2003) using 0.035M NaOH at pH 11 at 23°C (~70%).

Table 3.10. Protein content of soluble and insoluble fractions of CDC Candle pearling flour (20 g) obtained with 0.1M NaOH (initial concentration) and water at different temperatures and pH[1]

pH	Solvent	Temp (°C)	Soluble fraction		Insoluble fraction		% of protein not solubilized	Recovery of total Protein (%)
			Weight (g)	Protein (% w/w, d.b)	Weight (g)	Protein (% w/w, d.b)		
6	water	23	4.06	20.21[a]	13.26	12.22[a]	43.79	55.47
6	water	55	4.21	17.86[a]	12.86	12.78[a]	44.43	54.23
6	water	90	3.98	13.58[b]	13.09	12.59[a]	44.55	48.66
11	NaOH	23	9.42	17.87[a]	8.77	9.46[b]	22.42	57.40
11	NaOH	55	7.10	15.27[b]	10.10	9.62[b]	26.24	45.02
11	NaOH	90	10.95	14.20[b]	2.44	6.78[c]	24.47	35.94

All extractions and analysis of each sample were performed in duplicate.

[a-c]Means with different letters within a column are significantly different ($p \leq 0.05$).

Considering that the protein content has been high after the pentosans extraction, different methodologies have been performed in an effort to remove the excess of residues generated during the extraction affecting the purity of the samples. Delcour et al (1999), using a clay purification treatment, was able to remove practically all the secondary components found in the extracts (protein, 0.0%). In this study, a high content of protein was still found in the samples, affecting the purity of the final product.

3.3.2. Color

The color is an important physical property of food products since it is the first characteristic that a consumer assesses visually. During new product development, sensory analyses are performed to evaluate the impact of color on the acceptability of precuts by consumers. Therefore, the impact of any food ingredient on the color of a food product is critical. Thus, the color of the soluble and insoluble fractions obtained in this study was evaluated to assess their potential as fibre ingredients.

The color values determined by the Hunterlab color meter of the CDC Candle pearling flour fractions obtained under different conditions were presented in Tables 3.11-3.13. Since *L* value is an indication of the degree of whiteness, it was assessed as the most important color parameter for the purpose of this study. Therefore, only *L* values were statistically analyzed. The color values for starting material (control) were 91.66 (*L*), -1.00 (*a*) and -0.20 (*b*).

Table 3.11. Hunter color values (*L, a, b*) of soluble and insoluble fractions of Candle pearling flour obtained at different pH and initial alkali concentrations[1]

pH	Initial NaOH Concentration (M)	Soluble fraction			Insoluble fraction		
		L	*a*	*b*	*L*	*a*	*b*
9	0.04	55.32^{c}	3.07	7.41	53.57^{e}	4.01	12.24
9	0.07	49.10^{e}	3.77	7.46	54.51^{d}	4.17	12.52
9	0.10	44.92^{f}	4.38	8.13	51.86^{f}	3.47	10.72
9	0.15	80.71^{a}	0.73	6.81	43.87^{i}	3.25	10.40
9	0.20	61.10^{b}	3.57	7.36	41.60^{j}	4.11	9.57
11	0.04	61.32^{b}	2.87	8.22	57.56^{a}	3.60	14.03
11	0.07	48.31^{c}	3.40	7.89	57.14^{b}	3.11	14.69
11	0.10	43.01^{f}	2.22	4.52	55.31^{c}	4.07	12.70
11	0.15	52.25^{d}	5.23	9.35	49.28^{g}	4.26	12.61
11	0.20	55.27^{c}	5.26	9.24	46.35^{h}	4.49	12.07

[1]Values are means of two determinations. Hunter color values: *L* (0 black, 100 white), *a* (⁻green ⁺red) and *b* (⁻blue, ⁺yellow)

[a-j]Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.12. Hunter color values (*L, a, b*) of the soluble and insoluble fractions of Candle pearling flour obtained at different pH and high initial alkali concentrations[1]

pH	Initial NaOH Concentration (M)	Soluble fraction			Insoluble fraction		
		L	*a*	*b*	*L*	*a*	*b*
9	0.2	36.46[f]	3.62	4.13	43.82[c]	5.21	9.56
9	1.0	43.40[c]	6.74	6.30	50.67[b]	4.06	7.08
9	2.5	41.34[d]	3.69	3.91	54.64[a]	2.50	5.06
11	0.2	37.84[e]	6.05	7.38	41.08[d]	4.74	11.11
11	1.0	57.80[a]	6.51	10.01	41.35[d]	5.49	10.08
11	2.5	45.50[b]	5.54	6.79	55.58[a]	3.15	7.40

[1]Values are means of two determinations. Hunter color values: *L* (0 black, 100 white), *a* (⁻green ⁺red) and *b* (⁻blue, ⁺yellow)

[a-f]Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 3.13. Hunter color values (*L, a, b*) of the soluble and insoluble fractions of Candle pearling flour obtained at different temperature and pH conditions[1]

pH	Solvent	Temp (°C)	Soluble fraction			Insoluble fraction		
			L	*a*	*b*	*L*	*a*	*b*
6	water	23	61.11[a]	2.15	16.90	58.35[a]	1.86	11.09
6	water	55	62.61[a]	4.00	20.85	54.91[a]	2.25	10.06
6	water	90	67.15[a]	2.06	17.55	57.04[a]	2.33	11.10
11	NaOH	23	48.31[c]	7.47	17.73	57.18[a]	3.44	19.04
11	NaOH	55	37.36[b]	8.75	13.21	49.91[a]	6.06	16.28
11	NaOH	90	36.85[b]	8.52	12.72	43.99[a]	7.18	15.24

[1]Values are means of two determinations. Hunter color values: *L* (0 black, 100 white), *a* (⁻green ⁺red) and *b* (⁻blue, ⁺yellow)

[a-c]Means with different letters within a column are significantly different ($p<0.05$).

In the first set of extractions (Table 3.11), there was a significant drop ($p \leq 0.05$) in the *L* value of insoluble fraction with an increase in alkali initial concentration, reaching 41.60 and 46.35 at 0.2M NaOH at pH 9 and 11, respectively. However, there was no such consistent trend for the soluble fractions, since the *L* value first decreased and then increased with an increase in the initial alkali concentration.

In the second set of extractions (Table 3.12), both pH and initial alkali concentration had a significant effect ($p \leq 0.05$) on the whiteness of the soluble and insoluble fractions. When the alkali concentration was increased (0.2M, 1M, 2.5M), there was a significant decrease in the *L* value of the soluble fraction ($p \leq 0.05$) and a significant increase ($p \leq 0.05$) in the *L* value of the insoluble fraction.

In the third set of extractions (Table 3.13), the different pH (6/11), solvents (water and 0.1M NaOH) and temperatures (23°C, 55°C, 90°C) had a significant effect ($p \leq 0.05$) on the *L* value of the soluble fraction but there was no significant effect ($p > 0.05$) on the *L* value of the insoluble fraction.

Based on the results presented, it is believed that at the different basic pH conditions and high initial alkali concentrations (NaOH) used, a possible combination of the oxidation of phenolic acids and the Maillard reactions (between reducing sugars, glucose/maltose and amino acids or proteins, producing melanoidines) could be responsible for the color variations observed (Potus 1993).

3.4. Conclusions

The findings have shown that more research is needed for a better understanding of the associations of the components in the pearling flour. The distribution of the pentosans into the insoluble fraction was confirmed with the high percentage of pentosans not being solubilized after the extraction at low initial alkali concentrations. It is important to note that high initial alkali concentrations decrease the contents of pentosans, β-glucan and proteins in the insoluble fractions, increasing their solubilization and contents in the soluble fractions.

The color changes of the samples were visible, with the samples getting darker throughout the whole extraction procedure. It is necessary to continue further analysis of the effect of pH, alkali conditions and solvent interactions with the pearling flour components to better understand the reactions responsible for these changes.

3.5. References

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4. ENZYMATIC AND MECHANICAL TREATMENTS FOR CONCENTRATION OF PENTOSANS FROM BARLEY PEARLING FLOUR[2]

4.1. Introduction

Based on the extractions carried out at different pH, temperature and alkali concentration conditions, it was found that the pentosans are mainly concentrated in the insoluble fraction of the barley pearling flour (Chapter 3). Therefore, different methods to improve the method of isolation should be performed and adopted. It is believed that the distribution of pentosans between the soluble and insoluble fractions depends on the strong linkages between the components of the outer layers of the grain.

Although the major structural differences between the main non-starch polysaccharides (NSP) components in barley and other cereals have been reported (Fincher and Stone 1986) the literature is still lacking information. For example, the association between pentosans and other components (proteins, phenolic acids, β-glucan and starch) is not fully understood and thus more research is needed (Cui et al 1999).

Traditional methods to isolate water-insoluble cell wall material have been reported with mixed results. Procedures involving centrifugation of flour suspensions (D'Appolonia and MacArthur 1975) resulting in sludge with further purifications have been used. Alternative isolation methods have also been performed, including wet-sieving and ultrasonication in aqueous ethanol (Hromádková and Ebringerová 2003) or removal of starch and protein by organic solvents (Izydorczyk et al 1998). However, the

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[2] A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

application of these and other procedures has resulted in large amounts of protein and/or starch collected, which are by-products requiring further development.

Ultrasound treatment can be one approach to enhance the concentration of pentosans. Ultrasound treatment involves the use of high frequency sound waves that travel 10 to 20 million cycles per second above 20 kHz (Anderson et al 1978). Ultrasound has been widely used in medicine and in the investigation of the properties of inorganic materials. However, to date, it has found little application in cereal processing. Ultrasonic methods can also be used to examine food and related systems (Ebringerová and Hromádková 1999).

The use of ultrasound treatments during the extraction of polysaccharides has been documented for various types of plant tissues with demonstrated improvements (helping the disassociation of cell wall) in the isolation of some components (Hromádková and Ebringerová 2003). Therefore, if ultrasound treatment were to be applied to the pearling flour it may be possible to disrupt its structure and release or facilitate the separation of their components. There are reasons to believe in the existence of a tight association between cellulose, pentosans, β-glucan and protein within the bran.

The use of enzymes for the purification or removal of components during the isolation or hydrolysis of NSP has been used in various studies reported in the literature (Fincher and Stone 1986; Izydorczyk et al 1998; Cui and Wood 1999 and Delcour 1999). For example, Cui and Wood (1999) carried out the isolation of NSP from wheat bran with a combination of alkali solutions and thermal stable α-amylase (from *Bacillus subtilis*) to remove the residual starch (~14%) without any loss of NSP (~9%). Also, the

extraction of NSP from barley grain was reported with the help of porcine pancreas α-amylase under controlled conditions (pH 6.5, 35°C) for 24 hr with no trace of starch in the final extract (Izydorczyk et al 1998). In similar studies, the use of proteases (alcalase, neutrase, fungal protease) for protein removal from residual extracts has also been performed with good results for final extracts (Faurot 1995; Izydorczyk et al 1998). Enzymes have acquired an important role in the isolation of NSP. The use of enzymes should be considered in further research to develop and improve methodology for NSP extractions.

The overall objective of this study was to improve the concentration of insoluble pentosans using different techniques. Ultrasound and a combination of enzymes (α-amylase and fungal protease) were used in this study in an effort to disrupt the associations that could be affecting the separation of insoluble pentosans from the other components in the pearling flour and at the same time reduce the presence of residual protein and starch. Ultrasound and enzyme treatments were applied under specific conditions of temperature, pH and different solvents. The specific objectives were:

1. to perform extractions of barley pearling flour under different conditions of solvent, pH, enzymatic treatment or ultrasound power,
2. to analyze the effect of enzymatic treatments or ultrasound on the composition (pentosans, β-glucan and proteins) of soluble and insoluble fractions obtained during the extractions,

3. to evaluate the impact of alkali conditions, enzymatic treatments or ultrasound on the structure of soluble and insoluble fractions using scanning electron microscopy, and
4. to evaluate the color of soluble and insoluble fractions obtained to elucidate the impact of phenolic acids.

4.2. Materials and Methods

4.2.1. Materials

CDC Candle barley grains, a hull-less waxy barley variety, were provided by Agricore United (Calgary, AB). The 19% Pearling Flour (PF) fraction obtained by Lekhi (2003) was previously stored at 4°C and used as the starting material in this study. Prior to use in the extraction experiments, any clumps present in the pearling flour sample were manually broken on a 0.5 mm screen to ensure that the whole sample passed through. Sodium hydroxide, 98.5% of purity, ethanol 100% purity, and HCl, 37.5% purity, were obtained from Sigma Chemical Co (St. Louis, Mo).

The enzymes used were α-amylase (fungamyl 800 L) obtained from Novozyme, Novo Nordisk A/S (Bagsvaerd, Denmark), and fungal protease (400,000 HUT – Hemoglobin Units) from Deerland Enzymes (Kennesaw, GA). The conditions of pH and temperature for optimal activity of α-amylase (fungamyl 800L) were identified as 5.5 to 7 and 50 to 60°C, respectively, by its manufacturer. For fungal protease, the conditions of

pH and temperature for optimal activity were identified as 5 to 7 and 50 to 65°C, respectively, by its manufacturer.

4.2.2. Extraction procedure

The extraction protocols used are presented in Figures 4.1 and 4.2. Two sets of extractions were performed. The first set included individual treatments with enzymes and ultrasound performed at 55°C and pH 5.5 for 1 hr. The second set was a combination of enzymatic and ultrasound treatments at 55°C and pH 5.5 for 1 hr and different solvents (water and 0.0375M NaOH). Enzymes were applied after a pH adjustment in all samples prior to extraction for 1 hr at each step in the first and second set of extractions. Extraction carried out with water and no enzyme or ultrasound treatment served as control.

Extraction set one (Fig. 4.1) was divided into three groups: In Group 1, barley pearling flour samples (70 g) were dispersed in 1:25 (w/v) volumes of water, ultrasound treatment (Sonic 300 Dismembrator, Artek System Co, Farmingdale, NY) was applied for 15 min at 90% power, pH was re-adjusted and maintained at 5.5 for the entire extraction period of 1 hr with constant stirring in a water bath at 55°C. In Group 2, barley pearling flour samples (70 g) were also dispersed in 1:25 (w/v) volumes of water but no ultrasound was applied; pH was re-adjusted and maintained at 5.5 for the entire extraction period of 1 hr with the addition of 0.5 mL of α-amylase with constant stirring in a water bath at 55°C.

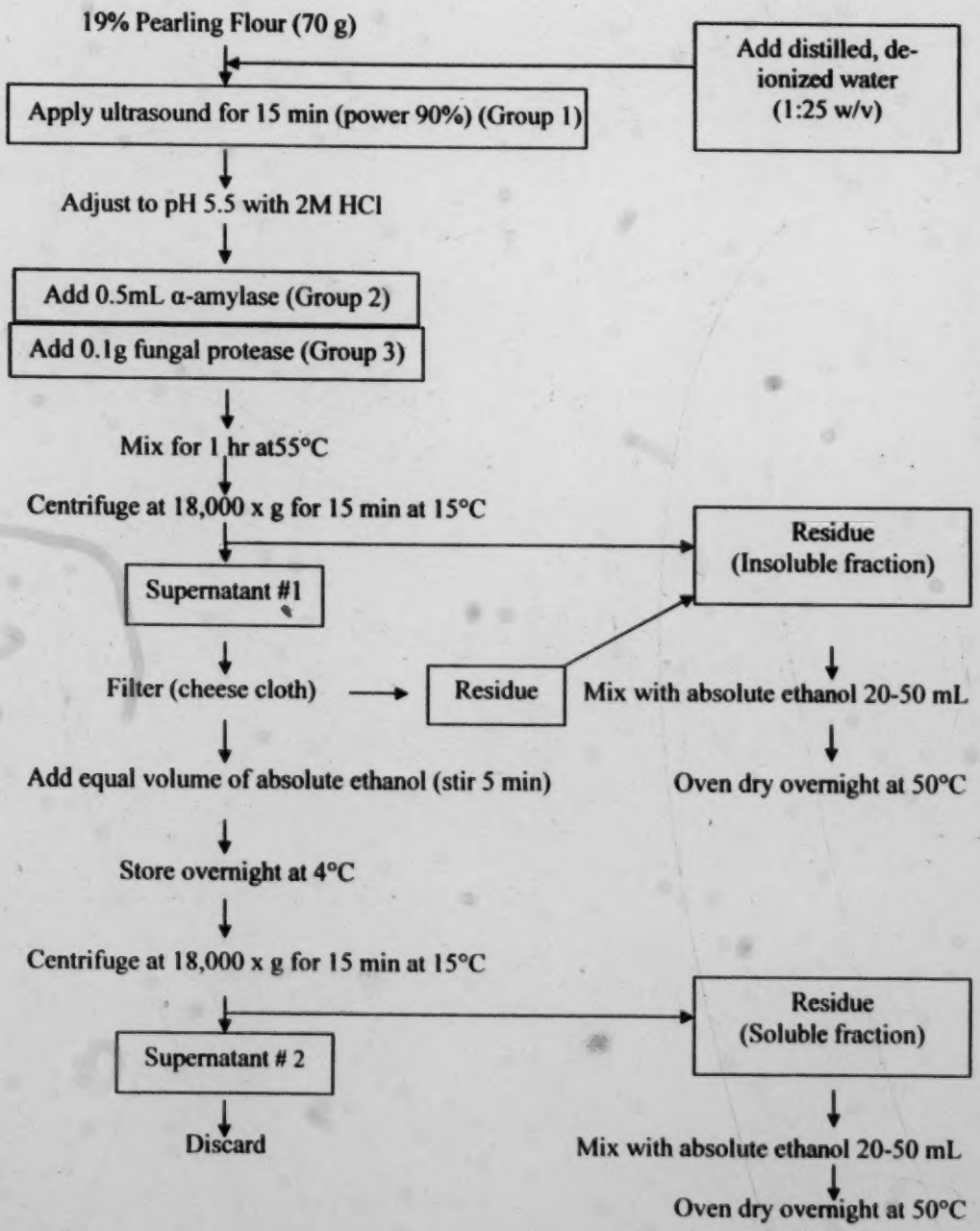
19% Pearling Flour (70 g)
Add distilled, de-ionized water (1:25 w/v)
Apply ultrasound for 15 min (power 90%) (Group 1)
Adjust to pH 5.5 with 2M HCl
Add 0.5mL α-amylase (Group 2)
Add 0.1g fungal protease (Group 3)
Mix for 1 hr at55°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Insoluble fraction)
Supernatant #1
Filter (cheese cloth)
Residue
Mix with absolute ethanol 20-50 mL
Add equal volume of absolute ethanol (stir 5 min)
Oven dry overnight at 50°C
Store overnight at 4°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Soluble fraction)
Supernatant # 2
Discard
Mix with absolute ethanol 20-50 mL
Oven dry overnight at 50°C

Figure 4.1. Flow diagram for the recovery of soluble and insoluble fractions from 19% pearling flour of CDC Candle barley using different enzymatic or ultrasound treatments.

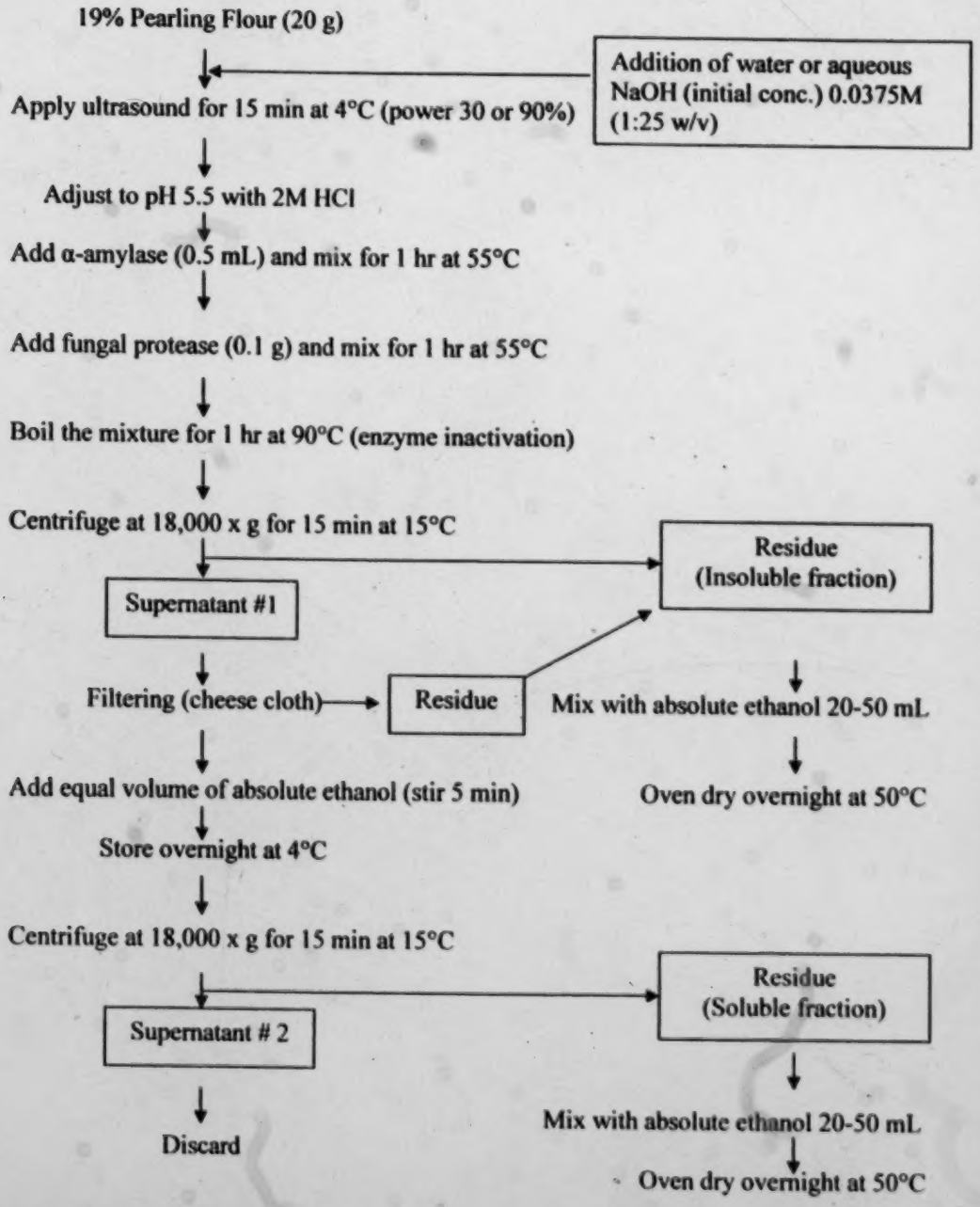
19% Pearling Flour (20 g)
Addition of water or aqueous NaOH (initial conc.) 0.0375M (1:25 w/v)
Apply ultrasound for 15 min at 4°C (power 30 or 90%)
Adjust to pH 5.5 with 2M HCl
Add α-amylase (0.5 mL) and mix for 1 hr at 55°C
Add fungal protease (0.1 g) and mix for 1 hr at 55°C
Boil the mixture for 1 hr at 90°C (enzyme inactivation)
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Insoluble fraction)
Supernatant #1
Filtering (cheese cloth)
Residue
Mix with absolute ethanol 20-50 mL
Add equal volume of absolute ethanol (stir 5 min)
Oven dry overnight at 50°C
Store overnight at 4°C
Centrifuge at 18,000 x g for 15 min at 15°C
Residue (Soluble fraction)
Supernatant # 2
Discard
Mix with absolute ethanol 20-50 mL
Oven dry overnight at 50°C

Figure 4.2. Flow diagram for the recovery of soluble and insoluble fractions from 19% pearling flour of CDC Candle barley in water or 0.0375M NaOH using combined ultrasound and enzymatic treatments.

The treatment of Group 3 was similar to that of Group 2 except that 0.1 g of fungal protease was added instead of α-amylase. At the end of the 1 hr extraction period in each group of experiments, the flour slurry was centrifuged (Model J2-21 Beckman Instrument Co., Mississauga, ON) for 15 min at 18,000 x g at 15°C to remove solids (insoluble fraction). The insoluble fraction was mixed with 20-50 mL of pure ethanol and then dried in an isothermal oven (Model 655G Fisher Scientific, Germany) at 50°C for 20 hr (overnight).

In the second set of extractions (Fig. 4.2), barley pearling flour samples (20 g) were dispersed in 25 volumes of water or 0.0375M NaOH. Ultrasound treatment was applied for 15 min at 2 different power settings (30 and 90%), pH was re-adjusted and maintained at 5.5 for the entire extraction period (3 hr) with constant stirring in a water bath at 55°C. α-Amylase (0.5 mL) was added to the slurry at the beginning of the first hour of extraction. After 1 hr, 0.1 g of fungal protease was added to the slurry while mixing was continued for another hour. For the final hour of extraction, the temperature was increased to 90°C (boiling conditions) to inactivate the enzymes. At the end of the 3 hr extraction procedure, the flour slurry was centrifuged (Model J2-21 Beckman Instrument Co., Mississauga, ON) for 15 min at 18,000 x g at 15°C to remove the insoluble fraction. The insoluble fraction was recovered, mixed with 20-50 mL of absolute ethanol and then dried in an isothermal oven (Model 655G Fisher scientific, Germany) at 55°C for 20 hr (overnight).

The supernatant (for each set of extractions), obtained from the centrifugation step, was decanted and filtered through a cheese cloth (with 100-200μm opening). Part of

the residue (insoluble fraction) was recovered here and combined with the residue previously collected (first centrifugation) and treated similarly as explained above. An equal volume of absolute ethanol was added to the supernatant (constant stirring for 5 min) to obtain a 50% ethanol solution, which was left undisturbed overnight at 4°C to precipitate the NSP (pentosans and β-glucan). The next day, the NSP were recovered by centrifugation at 18,000 x g at 15°C and the residue (soluble fraction) was mixed with 20-50 mL of absolute ethanol and left in the fume hood for 15 min. The residue (soluble fraction) was dried in an isothermal oven at 50°C for 20 hr (overnight).

Once the samples were dried (soluble and insoluble fractions), they were ground in a coffee grinder (Model KSM2 (4) Braun, DF, Mexico) for 2 min and then manually in a mortar and pestle until the particle size was small enough to pass through a 0.5 mm screen. Samples were collected and stored at room temperature for further analysis. Analyses were performed within the next 2 days following the extraction.

4.2.3. Composition analysis

Pentosans content determination was performed following the orcinol and HCl method of Hashimoto et al (1987) with modifications. These modifications consisted of increased dilution of the sample at the final step from 1 mL to 18 mL and substitution of the filtration step with microfugation (speed 10, 5 min). This method of pentosan content determination is based on acid digestion of the sample followed by neutralization, fermentation of hexoses with fresh baker's yeast and color development by pentoses,

with orcinol and ferric chloride which was determined spectrophotometrically. The β-glucan determination was done according to McCleary and Glennie-Holmes (1985) using the Megazyme assay kit (Megazyme Inc., Wicklow, Ireland). Protein content was determined using a Nitrogen analyzer (Model FP 428, Leco Corp., St. Joseph, MI) (%N x 6.25). Moisture content was determined using the AACC method 44-19 (AACC, 1982). All analyses were performed in duplicate.

Percentage of pentosans, β-glucan or protein not solubilized and the recovery of each of these components were calculated and reported. For example, for pentosans: Percentage of pentosans not solubilized = (g pentosans in insoluble fraction)*100/(g pentosans in starting material).

Recovery of total pentosans = (g pentosans in soluble fraction + g pentosans in insoluble fraction)*100/(g pentosans in starting material)

4.2.4. Scanning electron microscopy (SEM)

The barley pearling flour sample and the soluble and insoluble fractions obtained after treatments with water, aqueous NaOH (0.0375M), ultrasound and enzymes (amylase and fungal protease) were examined using a JEOL scanning electron microscope (Model JSM 6301FXV, JOEL, Ltd., Tokyo, Japan) with an acceleration voltage of 5 kV. Ground samples were mounted on semi-circular stubs with double sided carbon sticky tape and then sputtered with 100 Å of gold.

4.2.5. Color

Color measurements of the soluble and insoluble fractions obtained from 19% pearling flour CDC Candle barley were performed in duplicate using a LabScan XE Hunterlab color difference meter (Model D52-2, Hunter Associates Laboratory, Fairfax, VA). Color values for Hunter *L* (0 black, 100 white), *a* (-green, +red) and *b* (-blue, +yellow) were recorded. Calibration was performed using a white tile with known values of *L* (91.66), *a* (-1.00) and *b* (-0.20).

4.2.6. Statistical analysis

The extraction protocols at each combination of pH/solvent/enzymatic treatment/ultrasound were carried out in duplicate. The compositional analysis of each fraction obtained was also performed in duplicate. Analysis of variance of the results was done using General Linear Model (GLM) procedure of SAS Statistical Software, Version 8.2 (SAS Institute, 2002). The model for the first set of extraction data consisted of the main effects of enzymatic or ultrasound treatment and their interaction effects on pentosan, β-glucan and protein contents. The model for the second set of data consisted of the main effects of pH, solvent, the ultrasound power and their interaction effects. In both cases, multiple comparison of means was performed by Tukey's studentized HSD (Honestly Significant Difference) range test at $\alpha = 0.05$ level of confidence.

4.3. Results and discussion

CDC Candle barley pearling flour (19% PF) was obtained in a previous thesis research performed by Lekhi (2003). As reported previously (Table 3.1), the pearling flour contained 11.14% pentosans, 4.86% β-glucan, 24.81% protein and 18.64% starch. For the soluble and insoluble fractions obtained under different conditions in this study, only pentosan, β-glucan and protein contents were determined.

As a continuation of previous research focusing on the isolation of β-glucan (Temelli 1997; Burkus and Temelli 1998) and protein (Lekhi 2003), the focus of this study was to better understand the distribution of these components under conditions typically used for β-glucan and protein isolation but with the addition of enzymatic and ultrasound treatments.

4.3.1. Composition and yields of fractions (soluble and insoluble) obtained with enzymatic and ultrasound treatments

4.3.1.1. Pentosans

Two sets of extractions were performed. The first set of extraction was carried out at pH 5.5 using water as a solvent with different enzyme or ultrasound treatments and the results are presented in Table 4.1. The treatments applied had a significant effect ($p \leq 0.05$) on the pentosan content of the soluble and insoluble fractions.

Table 4.1. Pentosans content of soluble and insoluble fractions of CDC Candle pearling flour (70 g) obtained with water at 55°C, pH 5.5 and different enzyme or ultrasound treatments[1].

Enzyme or ultrasound	Soluble fraction		Insoluble fraction		% of pentosans not solubilized	Recovery of total pentosans (%)
	Weight (g)	Pentosan (% w/w, d.b)	Weight (g)	Pentosan (% w/w, d.b)		
Control[2]	4.21	8.91[a]	12.86	9.97[c]	57.55	64.35
α-Amylase	2.10	4.58[c]	35.30	14.38[b]	64.12	58.32
Protease	2.12	4.03[d]	25.16	15.77[a]	50.75	45.57
Ultrasound	6.88	5.02[b]	31.91	14.80[b]	60.37	57.18

[1]All extracted samples and analysis samples were made in duplicate. [2]Control: Water at 55°C and pH 6.
[a-d]Means with different letters within a column are significantly different ($p \leq 0.05$).

The pentosan content of the insoluble fraction obtained with protease treatment (15.77%) was significantly higher ($p \leq 0.05$) compared to those of insoluble fractions obtained with ultrasound (14.80%) and α-amylase (14.38%) treatments, which were similar ($p > 0.05$).

All of the treatments resulted in a significantly ($p > 0.05$) higher pentosan content in the insoluble fraction compared to the control. As expected, the soluble fraction contained a small amount of pentosans compared to the insoluble fraction due to the distribution of pentosans between the two fractions. This was also confirmed with the percentage of pentosans that did not solubilize (50.75-64.12%). For the soluble fraction, the ultrasound treatment presented the highest pentosan content (5.02%), and a significant decrease ($p \leq 0.05$) was registered when enzymatic treatments (α-amylase: 4.58% and protease: 4.03%) were applied. However, because the absolute difference between these results was small, there may not be a major advantage of one treatment over another from a practical point of view. The pentosan recoveries ranged from 45.57 to 58.32% when different treatments were applied (Table 4.1).

For the second extraction set (Table 4.2), different pH (5.5 and 11), solvents (water or NaOH) and the enzyme and ultrasound combination had a significant effect ($p \leq 0.05$) on the pentosan contents of soluble and insoluble fractions. The highest pentosan contents ($p \leq 0.05$) were registered in the insoluble fraction obtained with water at pH 5.5 and 90% of ultrasound power (21.11%) and pH 11 and 30% of ultrasound power (20.95%). Higher pentosan concentrations in the insoluble fraction were achieved when water was used as the solvent under similar conditions.

Table 4.2. Pentosan content of soluble and insoluble fraction of CDC Candle pearling flour (20 g) obtained with water or 0.0375M NaOH (initial concentration) at 55°C, different pH and enzymatic and ultrasound combination[1]

pH	Solvent	Enzyme	Ultrasound power (%)	Soluble fraction		Insoluble fraction		% of pentosans not solubilized	Recovery of total pentosan (%)
				Weight (g)	Pentosan (% w/w, d.b)	Weight (g)	Pentosan (% w/w, d.b)		
5.50	Water	amylase + protease	30	2.00	1.81^{d}	6.90	15.18^{c}	46.98	40.22
5.50	NaOH		30	2.00	0.53^{e}	6.57	13.93^{c}	41.14	33.79
5.50	Water		90	1.11	2.93^{b}	8.44	21.11^{a}	79.95	71.22
5.50	NaOH		90	0.86	4.04^{a}	9.83	18.72^{b}	82.61	74.62
11.00	Water		30	1.06	2.28^{c}	10.23	20.95^{a}	96.19	86.04
11.00	NaOH		30	1.12	3.01^{b}	8.86	18.52^{b}	73.65	66.64
11.00	Water		90	8.32	2.22^{c}	24.44	11.00^{d}	46.41	32.16
11.00	NaOH		90	7.59	2.66^{b}	25.56	13.55^{c}	55.86	34.28

[1] All extracted samples and analysis samples were made in duplicate.

[a-e] Means with different letters within a column are significantly different ($p \leq 0.05$).

In terms of the soluble fraction, the highest pentosan content was registered with 0.0375M NaOH at pH 5.5 and 90% of ultrasound power (4.04%). The percentage of pentosan not solubilized ranged from 41.14% to 96.19% (Table 4.2). The recovery values presented a big variation under specific conditions. It was possible to observe that those samples with higher pentosan contents corresponded to the highest recoveries, ranging from 71.22% to 84.04% (Table 4.2).

4.3.1.2. β-Glucan

The first set of extractions (Table 4.3) presented significant differences ($p \leq 0.05$) in the β-glucan contents of soluble and insoluble fractions. As expected, the highest β-glucan content was obtained in the soluble fraction with α-amylase treatment (24.29%), which was reduced significantly ($p \leq 0.05$) when protease (0.33%) and ultrasound (2.35%) were used. On the other hand, the insoluble fraction presented practically no difference ($p > 0.05$) in the β-glucan content (~2.5%) due to different treatments compared to the control. As expected, the percentage of β-glucan not solubilized was low (8.40-19.00%). These results were reflected in the total recovery of β-glucan being <30%, an indication of the substantial losses in the discarded ethanol fraction (Table 4.3). Therefore, it is necessary method to recover the β-glucan lost in the ethanolic residue possibly through different drying techniques.

Temelli (1997) reported extraction of Condor barley β-glucan under different temperature (40-55°C) and pH (7-10) conditions.

Table 4.3. β-Glucan content of soluble and insoluble fractions of CDC Candle pearling flour (70 g) obtained with water at 55°C, pH 5.5 and different enzyme or ultrasound treatments[1].

Enzyme or ultrasound	Soluble fraction		Insoluble fraction		% of β-glucan not solubilized	Recovery of total β-glucan (%)
	Weight (g)	β-glucan (% w/w, d.b)	Weight (g)	β-glucan (% w/w, d.b)		
Control[2]	4.21	8.95[b]	12.86	2.0[a]	21.80	46.60
α-Amylase	2.10	24.29[a]	35.30	2.1[a]	17.40	29.30
Protease	2.12	0.33[c]	25.16	1.4[a]	8.40	8.54
Ultrasound	6.88	2.35[c]	31.91	2.5[a]	19.00	22.81

[1]All extracted samples and analysis samples were carried out in duplicate. [2]Control: Water at 55°C and pH 6.
[a-c]Means with different letters within a column are significantly different ($p \leq 0.05$).

The values obtained in all conditions were superior to those obtained in this set of extractions with recoveries varying from 76 to 86%. However, the use of enzymes in this study, which was not considered by Temelli (1997), could affect the β-glucan content of the fractions obtained possibly due to β-glucanase side activity of the enzymes used.

The second set of extractions (Table 4.4) presented significant differences ($p \leq 0.05$) in the β-glucan content of the soluble and insoluble fractions. As expected, the β-glucan content of the insoluble fraction under the various conditions studied was low (1.00-3.01%). The soluble fraction was also very low in β-glucan (0.34-2.24%). The use of enzymes was a determinant factor that could explain the drastic reduction in the β-glucan content of the fractions obtained and the overall recovery. As explained previously, the enzymes used in this study were not pure and the presence of side activity, specifically β-glucanase and pentosanase should be considered since it is not possible to recover the small hydrolysis products of β-glucan by ethanol precipitation.

4.3.1.3. Protein

The first set of extractions (Table 4.5) presented significant differences ($p \leq 0.05$) in the protein contents of soluble and insoluble fractions. The highest protein content ($p \leq 0.05$) was registered in the insoluble fraction under α-amylase (22.63%) and ultrasound treatments (22.72%). However, there was no difference ($p > 0.05$) in the protein contents of the insoluble fractions obtained with or without protease treatment, even through a reduction was expected.

Table 4.4. β-Glucan content of the soluble and insoluble fractions obtained with water and 0.375M NaOH (initial concentration) at 55°C for 1hr, under enzyme treatment and different pH from 20 g of Candle pearling flour (19% pearled)[1]

pH	Solvent	Enzyme	Ultrasound power (%)	Soluble fraction		Insoluble fraction		% of β-glucan not solubilized	Recovery of total β-glucan (%)
				Weight (g)	β-glucan (% w/w, d.b)	Weight (g)	β-glucan (% w/w, d.b)		
5.50	Water	amylase + protease	30	2.00	0.34^{d}	6.90	1.98^{b}	1.98	2.55
5.50	NaOH		30	2.00	0.57^{c}	6.57	3.01^{a}	3.01	3.96
5.50	Water		90	1.11	1.55^{b}	8.44	1.91^{ab}	0.16	14.83
5.50	NaOH		90	0.86	1.47^{b}	9.83	1.90^{ab}	0.19	16.58
11.00	Water		30	1.06	1.45^{b}	10.23	2.01^{ab}	0.21	18.38
11.00	NaOH		30	1.12	1.36^{b}	8.86	2.17^{ab}	0.19	17.26
11.00	Water		90	8.32	2.24^{a}	24.44	2.33^{b}	0.19	15.67
11.00	NaOH		90	7.59	0.73^{c}	25.56	1.00^{c}	0.06	6.71

[1] All extracted samples and analysis samples were carried out in duplicate.

$^{a-c}$Means with different letters within a column are significantly different ($p \leq 0.05$).

Table 4.5. Protein content of soluble and insoluble fractions of CDC Candle pearling flour (70 g) obtained with water at 55°C, pH 5.5 and different enzyme or ultrasound treatments[1]

Enzyme or ultrasound	Soluble fraction		Insoluble fraction		% of protein not solubilized	Recovery of total protein (%)
	Weight (g)	Protein (% w/w, d.b)	Weight (g)	Protein (% w/w, d.b)		
Control[2]	4.21	17.86[b]	12.86	12.78[b]	44.43	54.23
α-Amylase	2.10	13.45[c]	35.30	22.63[a]	60.74	52.37
Protease	2.12	12.41[c]	25.16	14.90[b]	28.86	20.37
Ultrasound	6.88	20.49[a]	31.91	22.71[a]	55.79	56.12

[1]All extracted samples and analysis samples were carried out in duplicate. [2]Control: Water at 55°C and pH 6.
[a,b]Means with different letters within a column are significantly different ($p \leq 0.05$).

Comparing the values obtained in the soluble fraction with the insoluble fraction, the protein contents were lower, and again, when protease was used, there was a significant reduction in the protein content. The values for percentage of protein not solubilized (28.86%) and total protein recovery (20.37%) were substantially lower when protease was used, as shown in Table 4.5.

In the second set of extractions (Table 4.6), there was a significant variation ($p \leq 0.05$) in the protein contents of soluble and insoluble fractions. It was possible to obtain higher protein contents in the insoluble fractions with the highest level ($p \leq 0.05$) reached at pH 5.5 (31.44%) and 30% of ultrasound power using NaOH as the solvent. When the pH was increased to 11, there was a significant reduction ($p \leq 0.05$) in the protein content of insoluble fraction (~19%). In the soluble fraction, there was also a significant difference ($p \leq 0.05$) among the values presented. The percentages remained constant (~7%) at different conditions, except for pH 5.5, using water and 30% ultrasound power, which gave the highest protein content (10.53%) and pH 11 using NaOH and 30% ultrasound power, resulting in the lowest (5.99%) protein content. On the other hand, the total recovery of protein changed among all the conditions applied, ranging from 25.00% to 52.85% (Table 4.6).

4.3.2. SEM

Scanning Electron Micrograph (SEM) images of the 19% pearling flour of Candle barley and insoluble and soluble fractions obtained under different conditions are presented in Figures 4.3 and 4.4, respectively.

Table 4.6. Protein content of soluble and insoluble fractions obtained with water and 0.0375M NaOH (initial concentration) at 55°C for 1hr, under enzyme treatment and different pH from 20 g of Candle pearling flour (19% pearled)[1]

pH	Solvent	Enzyme	Ultrasound power (%)	Soluble fraction		Insoluble fraction		% of protein not solubilized	Recovery of total Protein (%)
				Weight (g)	Protein (% w/w, d.b)	Weight (g)	Protein (% w/w, d.b)		
5.50	Water	amylase + protease	30	2.00	10.53[a]	6.90	26.10[b]	60.74	40.48
5.50	NaOH		30	2.00	7.44[b]	6.57	31.44[a]	55.79	45.49
5.50	Water		90	1.11	6.78[b]	8.44	24.43[b]	42.08	35.03
5.50	NaOH		90	0.86	7.15[b]	9.83	24.24[b]	41.75	34.89
11.00	Water		30	1.06	8.21[b]	10.23	19.12[c]	28.86	26.61
11.00	NaOH		30	1.12	5.99[c]	8.86	18.84[c]	32.46	25.00
11.00	Water		90	8.32	6.71[b]	24.44	19.59[c]	46.04	52.85
11.00	NaOH		90	7.59	7.66[b]	25.56	19.45[c]	33.50	26.90

[1]All extracted samples and analysis samples were made in duplicate.

[a-c]Means with different letters within a column are significantly different ($p \leq 0.05$).

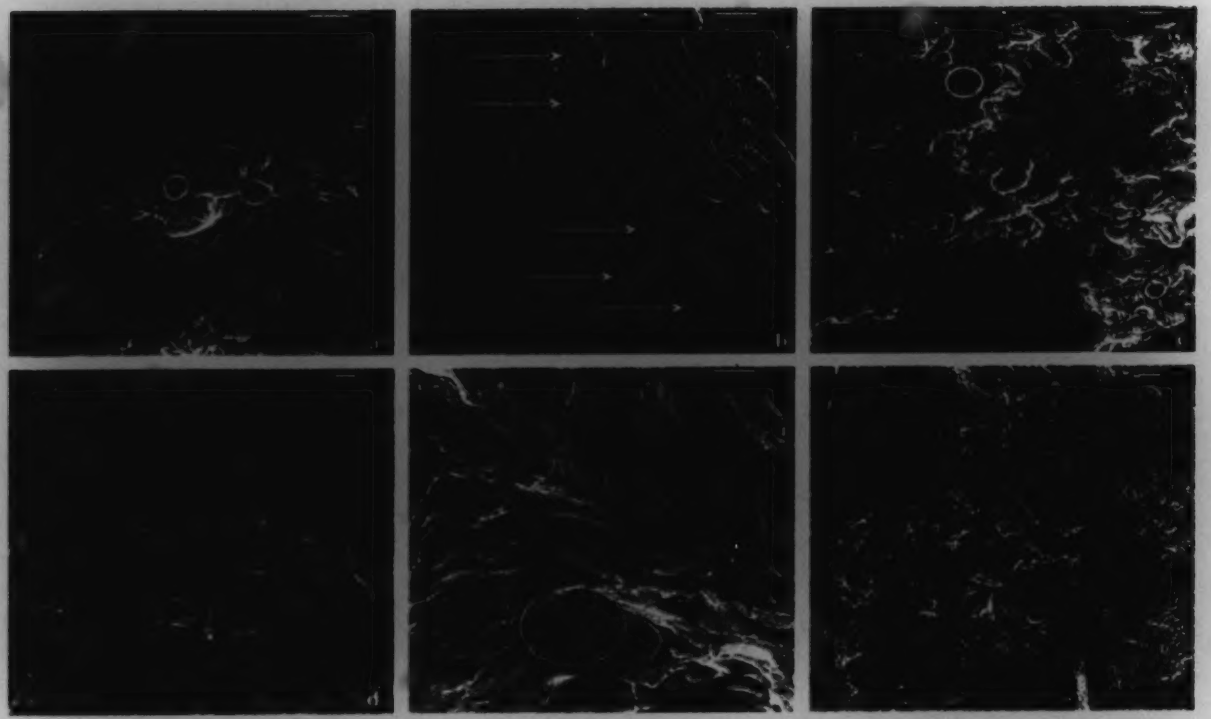

Figure 4.3. Scanning Electron Micrographs of insoluble fractions obtained with individual and/or combined enzymatic and ultrasound treatments at 1000X Magnification: a) CDC Candle 19% pearling flour, b) sample treated with α-amylase, c) sample treated with fungal protease, d) sample treated with ultrasound, e) sample treated with 0.1N NaOH and f) sample treated with 0.0375M NaOH and a combined enzymatic and ultrasound treatments.

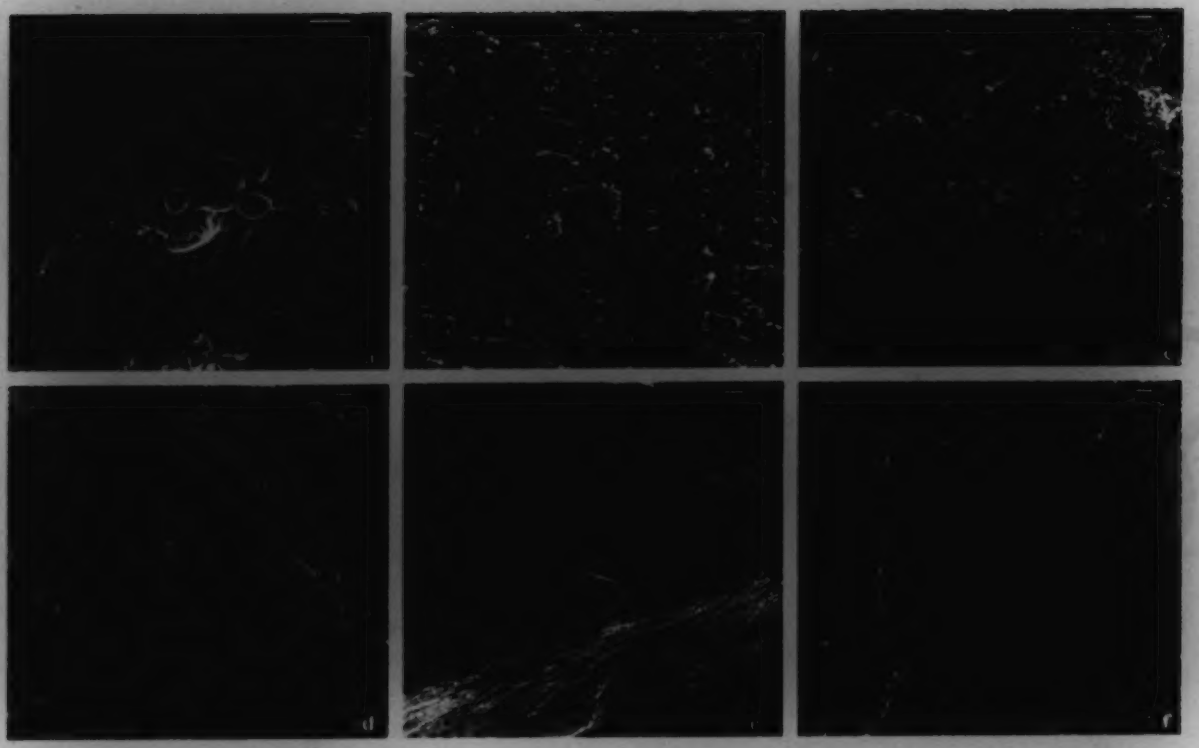

Figure 4.4. Scanning Electron Micrographs of soluble fractions obtained with individual and/or combined enzymatic and ultrasound treatments at 1000X Magnification: a) CDC Candle 19% pearling flour, b) sample treated with α-amylase, c) sample treated with fungal protease, d) sample treated with ultrasound, e) sample treated with 0.1N NaOH and f) sample treated with 0.0375M NaOH and combined enzymatic and ultrasound treatments.

Knowing that pentosans are concentrated mainly in the insoluble fraction, samples treated with enzymes (α-amylase and fungal protease) and ultrasound were analyzed and discussed in this section.

Differences were observed comparing the samples obtained under different treatment conditions (presented in section 4.3) and the original sample (CDC Candle barley 19% PF). The pearling flour sample presented a uniform structure of spherical granules of starch linked with a portion of what is believed to be a base of protein and polysaccharide network. The structure seems to be quite compact, which can provide an explanation for the subsequent results obtained after the solvent/enzyme/ultrasound treatments (Fig. 4.3).

The use of water as a solvent and α-amylase (Fig. 4.3b) during the concentration of the pentosans showed a clear difference between the insoluble fraction and the original sample. Fewer granules of starch were observed, showing the action of the enzyme over the surface of the bran sample. Although there is still some starch in the sample, their presence has been considerably reduced. Besides, it is also possible to see the protein and polysaccharide network, whose structure seems to be tighter than that previously observed (Fig 4.3b).

Using the same solvent and fungal protease, it was possible to test the effectiveness of the enzyme. The surface of the insoluble fraction presented a loose protein and polysaccharide network and starch granules (Fig. 4.3c). Comparing the samples treated with α-amylase (Fig. 4.3b) and protease (Fig. 4.3c), it is possible to observe the loosening of the protein network. It is still a tight association but comparing

the values presented in Table 4.5, the protein content was significantly decreased from 24% to almost 14%.

When ultrasound treatment was applied, the structure was clearly disrupted (loosened) as can be observed in Figure 4.3d. The number of starch granules was lower and the protein and polysaccharide network was broken. It is also possible to see the separation between components and the reduction of particles into the big structure.

The solvent was an important consideration during the extraction and concentration of pentosans. When aqueous NaOH was used at different alkali concentrations, it was possible to observe different behaviors. For example, 0.1M NaOH (initial concentration) at 55°C without any action of enzymes (α-amylase and protease) or ultrasound treatment presented a clear disruption of the protein network and a clear reduction in the starch granules in the sample (Fig. 4.3e), something that was not reflected in the pentosans content of the concentrate, remaining around 18% (Table 3.4). The sample treated with 0.0375M NaOH (initial concentration) and a combination of α-amylase, fungal protease and ultrasound treatments also presented a disruption of the structure of the protein and polysaccharide network, a reduction in the starch granules and a disintegration of the surface by the action of the enzymes (Fig. 4.3f). However, the pentosan content was not significantly improved (19%) (Table 4.2).

Figure 4.4 shows the SEM images for the soluble fractions obtained with different treatments following ethanol precipitation. Those samples treated with enzymes (Fig. 4.4b,c) exhibited a similar physical structure, which was different compared to those with

no enzymes (Fig. 4.4d,e). Enzyme hydrolysis product present in the soluble fraction recovered may impact the network of the ethanolic precipitate.

4.3.3 Color

Considering the potential use of the pearling flour fractions obtained as a fibre ingredient and the impact it may have on the color of the final products, color of the fractions was evaluated. The color values determined by the Hunterlab color meter of the soluble and insoluble fractions obtained from 19% PF CDC Candle barley were presented in Tables 4.7 and 4.8. The analysis showed a considerable difference between the whiteness values (*L*), but not much difference in the redness (*a*) and yellowness (*b*). *L* values were the only values statistically analyzed. The color values for starting material (control) were 91.66 (*L*), -1.00 (*a*) and -0.20 (*b*).

The *L* values for the samples analyzed were significantly affected ($p \leq 0.05$) by the different treatments used. The first set of extractions presented a significant difference ($p \leq 0.05$) in both fractions (soluble and insoluble). Whiteness of protease treated soluble fraction (62.37) was similar to that of the control but significantly higher ($p \leq 0.05$) than those of the soluble fractions obtained with α-amylase (37.22) and ultrasound (34.42) treatments. In the insoluble fraction, the most white ($p \leq 0.05$) sample was the control (54.91) followed by that obtained with ultrasound treatment (50.96) (Table 4.7).

The second set of extraction results (Table 4.8) presented significant differences ($p \leq 0.05$) in the *L* values of soluble and insoluble fractions.

Table 4.7. Hunter color values (*L, a, b*) of soluble and insoluble fractions of CDC Candle pearling flour obtained by enzyme and ultrasound treatments[1]

Enzyme or ultrasound	Soluble fraction			Insoluble fraction		
	L	*a*	*b*	*L*	*a*	*b*
Control[2]	62.61[a]	4.00	20.85	54.91[a]	2.25	10.06
α-amylase	37.22[b]	6.15	12.94	41.64[c]	2.12	8.06
protease	62.37[a]	1.26	8.21	43.85[b]	2.05	7.67
ultrasound	34.42[b]	5.27	11.04	50.96[a]	2.58	10.54

[1]Values are means of two determinations. Hunter color values: L (0 black, 100 white), a (⁻green, ⁺red) and b (⁻blue, ⁺yellow).
[2]Control: Water at 55°C and pH 6.
[a-d]Means with different letters within a column are significantly different ($p<0.05$)

Table 4.8. Hunter color values (*L, a, b*) of soluble and insoluble fractions of CDC Candle pearling flour obtained at different solvent, pH and ultrasound conditions[1]

pH	Solvent	Enzyme	Ultrasound power (%)	Soluble fraction			Insoluble fraction		
				L	*a*	*b*	*L*	*a*	*b*
5.50	Water	amylase + protease	30	44.12[c]	3.90	12.42	31.54[c]	5.08	10.23
5.50	NaOH		30	37.47[c]	3.40	9.91	31.04[c]	5.17	10.33
5.50	Water		90	65.98[b]	2.10	9.40	43.23[a]	3.76	11.48
5.50	NaOH		90	73.11[a]	2.16	8.85	49.73[a]	4.90	14.34
11.00	Water		30	69.65[b]	1.67	9.41	42.43[a]	3.63	11.00
11.00	NaOH		30	72.83[a]	2.36	9.36	43.80[a]	5.71	13.69
11.00	Water		90	65.76[b]	2.46	9.10	37.80[b]	4.55	10.39
11.00	NaOH		90	61.73[b]	3.12	8.94	33.67[c]	3.46	7.74

[1]Values are means of two determinations. Hunter color values: L (0 black, 100 white), a (⁻green, ⁺red) and b (⁻blue, ⁺yellow)

[a-c]Means with different letters within a column are significantly different ($p<0.05$).

Variations were registered in the insoluble fraction with mixed results ranging from 31.04 to 49.73. The darkest insoluble fractions were obtained at pH 5.5 with water or NaOH as a solvent and ultrasound power of 30% (31.54) as well as NaOH at pH 11 and ultrasound power of 90. This darkening may be due to the Maillard reaction of reduced sugars and proteins in alkali solutions. In the soluble fraction, the *L* values were higher than those in the insoluble fraction (37.47 to 73.11) (Table 4.8), with pH 5.5, water or NaOH as solvent and 30% ultrasound power again resulting in the darkest ($p \leq 0.05$) samples.

4.4. Conclusions

After the analysis of the results generated under the various conditions tested, a positive effect was observed on the concentration of pentosans when the ultrasound treatment was used. In the insoluble fractions, high alkali concentrations resulted in different pentosan and protein contents. However, for the recovery of β-glucan, the high alkali concentrations decreased the β-glucan content of the insoluble fractions, increasing their solubilization and β-glucan content of the soluble fractions as observed previously in Chapter 3.

Differences among the samples were found in the SEM images of the samples before and after the treatment. When the ultrasound was used, it was possible to see a loosening of the structure of the samples and in some cases, the amount of pentosans was relatively increased. The reduction in the concentration of pentosans recovered in the soluble and insoluble fractions could be attributed to the enzyme treatments used (α-amylase and fungal protease) as the activities of the side enzymes present should also be

considered. The manufacturers of the enzymes used confirmed the presence of β-glucanase and pentosanase. These enzymes could have side effects and thus a reduction in the pentosans and β-glucan contents were observed.

When the samples were treated with only one enzyme (α-amylase or fungal protease) or the mechanical treatment (ultrasound), the difference among the samples was perceived. The samples treated with ultrasound had a higher pentosan content in the insoluble fraction with a better disruption of their structure. However, further research is needed to have a better understanding of pentosans, their structures and improved ways to isolate them.

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5. CONCLUSIONS AND RECOMMENDATIONS

The importance of barley as a staple cereal in the world (ranking 4th), and its expanding utilization, not only as a major component in some processes (malting and brewing processes) but also as a food that provides a high fibre content, have opened new expectations in the search for diversification and innovation. Traditionally, in North America, barley has been used as a major feed component in the animal industry, but has seen limited use in human consumption. Bran part of cereals has been for the most part a component classified as waste by the industry relegated to be discarded based on the lack of capabilities to make use of it, even though it is being utilized to a very limited extent recently in high-fibre foods.

Studies have been performed to understand the usability of cereal components and also to take advantage of their structural composition. Important components detected in bran, especially non-starch polysaccharides (NSP) have been analyzed in recent years under different conditions (temperature, pH, enzymatic reactions, viscosity, structural interaction, and ultrasonication) in an effort to isolate these components and develop possible applications for human consumption.

This thesis research has investigated the distribution of one of the NSP components (pentosans), located in the outer layers of CDC Candle barley grains in an effort to isolate and concentrate them. The use of different solvents (water and aqueous NaOH), conditions of pH, temperature and initial alkali concentrations, as well as ultrasonication and enzymatic (α-amylase and fungal protease) treatments were selected

for further study based on past reports in the literature. It is important to highlight that this project has been based on previous studies within the barley fractionation research program at the University of Alberta, focusing on value-added processing of barley, especially β-glucan and protein extraction under different concentrations at different pH and temperature conditions. Isolation of pentosans has not been studied previously within this program.

In this thesis research, not only ways to separate the barley pearling flour components were studied and tested, but also, how these components were associated under different extraction conditions were analyzed. It was possible to recognize the strong interactions that exist between the components in pearling flour and the difficulty of separation due to their disassociation. Even though the results presented some variability after the application of different conditions, interesting questions were raised, opening *doors* for future research.

The findings demonstrated that a major portion of the pentosans remained insoluble under the majority of the conditions tested. High initial concentrations and pH resulted in the solubilization of pentosans, β-glucan and proteins to a greater extent. The use of enzymes and ultrasound treatment resulted in a significant ($p \leq 0.05$) improvement in the pentosan content of the insoluble fraction. When the enzyme and ultrasound treatments were used in combination, further enhancement of the pentosan content in the insoluble fraction was achieved, reaching a maximum of 21%. Scanning electron micrographs of the insoluble fractions obtained at different conditions demonstrated the loosening of the structure due to ultrasound treatments and removal

starch and proteins with the enzyme treatments. Another characteristic analyzed was the color of soluble and insoluble fractions during the extraction and concentration of pentosans. The presence and development of undesirable dark colors during the pentosans extraction were considered to be a result of different reactions, including oxidation of phenolic compound and Maillard reactions between sugars and proteins, combined with the action of acid or alkali conditions and different temperatures. The differences between procedures performed were significant and finding the optimum method to isolate pentosans will require further improvements to the experimental work.

It is recommended to explore the possibility of modifying the solvent (ethanol) used to precipitate the soluble fraction, increase the volume of absolute ethanol or use an alternative solvent (with similar characteristics and classified as a food grade solvent) in an effort to increase the recovery of the various components in the soluble fraction. It is also recommended to extend the precipitation times or test a combination of the time/precipitation effect. For the insoluble fractions, it is necessary to find a way to separate the residual components (protein and starch) from the pentosans to increase their content and at the same time keep it as a food grade product. It is also recommended to test different enzymes from the animal group (e.g. trypsin) during the extraction procedure to minimize any breakdown of NSP components due to side activity of other enzymes present in plant-based enzymes. It is recommended to continue with the application of ultrasound or test other mechanical options such as polytron (homogenizer), to improve the disruption of the physical structure of pearling flour to release major and minor components for an easier separation of pentosans. Also, it is

recommended to test the isolation and concentration of pentosans after reducing the particle size of the starting material. In addition to the results generated and presented in this study, it is important to mention that the objectives for the realization of this research are based on the idea to use this cereal grain and all its components, without underestimating the possibility of providing new options to a market that needs alternatives for human consumption. The importance of fibre consumption and the fact that the average North American consumption is well below the recommended amount are well recognized. As well, there is growing interest in the health benefits of fibre such as lowering glucose levels in the blood, cholesterol control, reduced the risk of heart disease and proper intestinal function.

The enhanced utilization of NSP is clearly an option for the future. There is growing market trends to provide healthy alternatives that can be included in everyday life. Further research is needed to find the best way to open and realize these opportunities.